"STUDIES IN THE HORMONE RELATIONS OF ROOT GROWTH"

by

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A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science in the University of London.

September, 1959.

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ACKNOWLEDGEMENTS.

I wish to express my deep gratitude and sincere thanks to Professor L.J. Audus for his supervision, advice and valuable criticism throughout this work.

My thanks are also due to Mr. M.C. Funnell for photographing the graphs and to Miss B. Seymour for typing the thesis.

ABSTRACT

The relationship between endogenous auxin levels and growth in roots has been studied. Paper chromatographic methods were adopted to separate the growth substances. Several assay methods (oat coleoptile and mesocotyl, wheat coleoptile and pea root section tests) were used for the detection and estimation of growth substances from chromatograms. Different patterns of endogenous auxin content could be obtained by following different purification and assay methods.

In Vicia faba roots acid auxins show a decline in concentration with increasing age of the seedlings. A fall in the growth rate of older roots has been correlated with the gradual disappearance or inactivation of growth substances. An increase in the concentration of IAA and total acid auxins in the early stages of growth could be related to the development of lateral meristems.

Different levels of auxin concentration are maintained in different parts of the root system, the highest being in the tap root tips. Growth substances in different parts of the roots (tap root tip, root stump and laterals) show complex changes in concentrations as the organs age. The possible bearing of such changes on root growth has been discussed.

The evidence indicates that synthesis of acid auxins in the lateral tips is inhibited by the presence of the tap root tips.

It was further demonstrated that geotropic stimulation triggers off certain enzymatic reactions which bring about an increased synthesis of an acid auxin (occurring in the IAA zone of chromatograms) in the root apices of broad beans. The maximum amount of the substance is produced in roots stimulated for 40 minutes - the concentration being lowered in a longer period of stimulation (60 minutes). This synthesis was in no way connected with the release of free auxins from the bound state.

Growth substances occurring in the water soluble fraction show spontaneous interconversion and all inhibit the growth of root sections (pea).

ABBREVIATIONS USED:

IAA	.. ..	Indolyl-3-acetic acid.
IAN	.. ..	Indolyl-3-acetonitrile.
IAc	.. ..	Indolylacetaldehyde.
IPyA	.. ..	Indole-3-pyruvic acid.
TTP	.. ..	Tryptophane.
TNH_2	.. ..	Tryptamine.
IBA	.. ..	Indolyl-3-butyric acid.
IPrA	.. ..	Indolyl-3-propionic acid.
IAAP	.. ..	Indolyl aspartile acetic acid.

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INTRODUCTION

(a) Problems related to hormone regulated root growth.

The field of our knowledge of plant growth substances has extended at a rapid and impressive pace. And yet, we feel the precise mechanisms which underlie their growth manifestations in plants are not properly understood. In consideration of the diverse nature of growth regulators that occur in the tissue, it is by no means easy to picture their delicate interplay, which ultimately controls and regulates the growth processes in plants. In this respect, the voluminous work on synthetic growth substances is not very informative. Moreover, the information about the endogenous level of auxin in plants that has been handed over to us by classical workers needs a careful re-examination, in view of the imperfect experimental methods followed in the early years of auxin research. Fortunately, it is possible to-day, with the development of refined techniques, to attack physiological problems related to growth with greater confidence.

The present investigation is devoted to a study of hormone relations of root growth. The main objective of the work was to obtain a straightforward and rational answer to problems related to endogenous balance of auxins in roots and its overall growth process. The nature of endogenous growth regulating factors in roots is still under dispute. Even the theory of supraoptimal concentration of auxin level in roots is not free from contradiction (Audus & Das, 1954). Again, very little is known about the distribution of growth substances in this part of the plant and the actual concentrations of auxins in the elongating part of the root, in the older part and laterals have never been clearly defined. The hormonal basis of geotropic responses of roots, though extensively investigated, still remains a very controversial subject.

However, researches in this line have been going on for a very long time. In the '70's and '80's of the last century a number of workers attempted to resolve the problems of root growth. The main object of their research was to find out whether decapitation of root tips retarded growth or was without effect. Weisner (1884) was first to observe that removal of tips can produce growth acceleration of roots. Cholodny's (1926) observation was a further support to this

fact. It was also noted that if sections more than one mm. were removed from the tip no acceleration could be observed (Bunning, 1928). However, our primary concepts of root growth and geotropism have mainly been derived from the work of Cholodny (1924, 1926 and 1928), Keeble, Nelson and Snow (1931) and other workers of the early 1930's.

In brief, the theory which crystallised from their work was that both root and shoot tips are the sites of hormone production or activation, and the substance they secrete has an inhibitory effect on roots and a stimulatory effect on coleoptiles. It was further deduced that auxin content in roots is supraoptimal, an increase causing retardation of growth. (Nielsen, 1930; Navez, 1933; and others).

The basic assumption about supraoptimal level of auxin in root, as deduced from decapitation experiments, has met with serious contradictions in recent years. Younis (1954) observed that decapitation of Vicia faba root tips, at 0.5, 1 or 2 mm. behind the root apex did not accelerate the growth of the stump. He further noted that the growth of the stumps reheaded with their own tips was similar to that of unreheaded stumps. HUMPHRIES? Hempshries (1958) reported that removal up to 50% of the roots of barley and rye has no effect on the growth rate which is the same as in the intact plant. Negative response of Pisum roots to decapitation treatment has also

been recorded in earlier investigations (Gorter, 1932; Ref. from Went and Thimann, 1937).

When indoleacetic acid was established as one of the naturally occurring growth hormones extensive work was carried out to study its effects on root growth. A typical concentration-response curve of this substance could be divided into two parts: in the first part, at higher concentrations inhibition, and in the second part at lower concentrations a significant promotion of growth. This phenomenon has been observed by various workers with root sections or intact roots. (Bonner and Koepli, 1939; Naylor and Rappaport, 1950; Moewus, 1949; Pohl, 1952; Thimann, 1952; Audus and Garrard, 1953; Roberts and Street, 1955; Lahiri, 1955; and others).

The stimulation of root growth by addition of auxin is a matter of considerable theoretical importance in view of the accepted idea of endogenous level of auxin. Audus and Garrard (1953) observed as much as 35% growth stimulation of pea root sections at 10^{-11} M concentration of indoleacetic acid. However, there are views that such stimulations at very low concentrations of indoleacetic acid are related to "adaptation" and aftereffect (Åberg, 1957), or that these stimulations are small and scarcely reproducible (Burström, 1957). But it is more or less an established fact that consistent promotion of growth could be observed at low

concentrations of indoleacetic acid (Macht and Grumlein, 1937; Moewus, 1949; Ashby, 1951; Pohl, 1952; Audus and Das, 1955; Roberts and Street, 1955; and others). Thimann (1937) suggested in this connection that roots differ from stems in having a much lower optima for the effect of growth substances upon elongation. But so long as the exact chemical natures of the naturally occurring growth substances in roots are not indisputably established it would be difficult to support or reject the classical hypothesis.

A more profitable way of investigation would be to collect information about the native auxins of roots. The preliminary work in this field started from investigations related to tropic phenomenon of this organ. Keeble, Nelson and Snow (1929) demonstrated that a geotropically stimulated root tip when stuck onto the stump of an unstimulated root can bring about curvature. By gelatin diffusion method Hawker (1932) showed that a greater positive curvature can be induced in an unstimulated root of Vicia faba by hormones extracted from lower halves of a geotropically stimulated root tip, than by hormones extracted from upper halves of the same root tip. This was in direct support of Cholodny (1924) and Went's (1927) theory. More information was gathered in course

of time about the endogenous growth substances in roots. Thimann (1934) reported that auxin concentration would decrease with increasing distances from tip. He also noted that considerable amount of auxin could be extracted in chloroform, from stump 20mm. away from the tip. Gorter (1936) however observed that most of the growth substances are confined to the tip and after 6mm. no growth activity could be observed. From a study of endogenous growth substances by paper chromatographic method Kefford (1955) noted that root auxins do not change in quality or quantity in regions 0-20mm. and 20-40mms. from the tip. Pilet (1958), following a similar technique, has demonstrated that in Lens roots all three growth substances gradually fall in concentration with increasing distance from the tip. Previously (1951), he made a similar observation on total auxin content by the agar diffusion method. These pieces of information are, unfortunately, very contradictory.

Recent chromatographic investigations on root auxins have shown that there are promoters and inhibitors other than indoleacetic acid present in this tissue. Bennet-Clark and Kefford (1953), and Kefford (1955) observed three growth substances in the acid fraction of their extract. Besides

indoleacetic acid, they also observed "accelerator α" running before indoleacetic acid and "inhibitor β" coming after indoleacetic acid on their chromatogram. It was further observed that accelerator α produced promotion in both root and coleoptile assay, and indoleacetic acid promoted coleoptile growth and inhibited root growth, and inhibitor β acted as inhibitor in both tests. Audus and Thresh (1956) detected two inhibitors besides indoleacetic acid in the acid fraction of pea root extracts and one inhibitor in the neutral fraction. They carried out the investigation with a new assay method with root sections (Audus and Thresh, 1953). In wheat roots Alexander (1953) was able to demonstrate the presence of accelerator α, indoleacetic acid and inhibitor β. Extensive information has recently been collected about these and other naturally occurring growth substances (Gordon, 1954; Bentley, 1958).

The chemical nature of all the endogenous auxins has not yet been established. In relation to roots the situation is even more problematical. Stowe and Thimann (1954) suggested that accelerator α might be 3-indolepyruvic acid. It has been contradicted by Housley and Bentley (1956). Again in all probability it is not identical to indoleaspartile acetic acid. (Dr. Wheeler's work mentioned by Bennet-Clark et al. 1959)

The physiological role of indoleacetic acid in roots is rather ambiguous from direct (Bennet-Clark et al. 1959) and indirect (Audus and Shipton, 1952; Street, 1954) evidences. However, consistent growth activity at the indoleacetic acid zone of the chromatogram has been observed in the acid fractions of root extracts so far investigated. The inhibitor β of potato has been subjected to critical examination by Housley and Taylor (1958). They observed that growth activity of this zone could not be related to a particular compound, but it is a complex mixture of aliphatic acids. It is tempting to believe that inhibitor β of roots also has a similar constitution.

A number of growth active compounds have also been known to occur in the water soluble fraction (Britton et al., 1956; Audus and Gunning, 1958). But their physiological roles within the root tissue is yet unknown. However, from studies on shoots, considerable information has been collected about growth regulating mechanisms of certain acid and neutral auxins (Blommaert, 1954; 1955; Hemberg, 1958a, 1958b, 1958c; Phillips and Wearing, 1958a, 1958b; Galun, 1959). In this respect, our knowledge of root auxins is very inadequate.

We have again very little information about the actual amounts of growth substances that occur in roots. Audus and Thresh (1956) observed about 1 µgm/kgm of indoleacetic acid (or the substance that occurred at the indoleacetic acid region of their chromatogram) in the acid fraction of pea root extract. Moewus et al. (1952) in Lepidium and Pilet (1951) in Lens observed much higher concentrations (in the range of 10^{-3}M). Audus and Gunning (1958) observed 10 µgm/hgm indoleacetic acid eq. activity in the compounds occurring in the water soluble fraction. Aberg's (1957) criticism was that, in view of observations made by certain authors about very high concentrations of growth substances in roots, it is difficult to understand why further addition of indoleacetic acid should promote growth.

However, it has been shown by Andreae and Good (1955) that exogenous indoleacetic acid is largely converted to indoleacetylaspartic acid in the tissue. Apart from this, it has also been shown that indoleacetic acid is incorporated into a protein complex by pea root tips (Siegal and Galston, 1953). Parallel to these, indoleacetic acid-oxidase systems would also be acting. Introduction of indoleacetic acid in the root tissue can trigger off diverse reactions but it does not

enlighten us about endogenous patterns of growth regulators. However, it may be mentioned in this connection that indoleacetic acid induced peroxidase appears to be of great importance in cellular differentiation of Vicia faba roots (Jensen, 1955).

In view of doubts expressed about the presence of indoleacetic acid in roots we shall have to reconsider the theory of adoptive formation of indoleacetic acid-oxidase (Galston and Dalberg, 1954) and its physiological significance. Pilet and Galston (1955) observed that the capacity of root cells to destroy indoleacetic acid increases with age and similarly indoleacetic acid-oxidase activity increases as one goes from tip to older part of a root. They also postulated that a DCP-like factor which can control biochemical process of auxin destruction would be the true "ageing factor" of roots.

On the basis of this finding Pilet's recent observations (1958) fit in very well. But the supraoptimal level of auxin in older roots and the suboptimal concentration in young roots as he suggested in earlier work (1951) would seem rather confusing. He suggested previously that in older roots high concentration of endogenous auxin inhibits, or decreases, the growth rate, and in younger roots promotion by indoleacetic

acid treatment could be observed due to its suboptimal concentration. In relation to ageing of cress roots Moewus and Moewus (1952) observed that indoleacetic acid in the short roots (5-6 mm. long) is about the double that of the long roots (20-25 mm. Long). The relationship of ageing of root and its balance of auxin is another enigma in relation to our present study. Street and his co-workers have tried to study the problem of ageing in roots from a different angle. From investigations on excised root culture Street (1954) concluded that indoleacetic acid may not be a primary controlling factor in ageing of roots. He further hypothesised that retardation of growth and cessation of meristematic activity in excised tomato roots is due to the accumulation at the apex of some natural hormone to a critical supraoptimal concentration.

The physiological relationship of lateral roots and the main axis is again very obscure. Since the observation of Zimmerman and Hitchcock (1935), that lateral root formation can be induced by the application of indoleacetic acid or by root decapitation, extensive investigations (Thimann, 1936; Torrey, 1950, 1952, 1956; and others) have been carried out in this field. It has been thought that an unidentified substance other than auxin is necessary for lateral formation

in pea roots, but the substance becomes active within the root under the influence of auxin (Torrey, 1950). We have also some information about the complex nutrient requirements of lateral initiation (Torrey, 1956), but we know nothing about the endogenous levels of auxins in the laterals and lateral meristem. The state of auxin adjustment which increases lateral formation and lateral growth in the absence of tap root tips is still enigmatic. However, the investigations of Libbert (1954, 1955, 1956) have produced some evidence of an inhibitor precursor in pea roots which interacts with indoleacetic acid to produce an ether soluble, neutral correlative inhibitor, which in turn inhibits lateral root production.

Geotropic phenomenon is another controversial problem in relation to root growth. According to Cholodny-Went's theory the geotropic response is due to a redistribution of internal auxin. Supporting evidences have also been furnished by Hawker (1932) and Larsen (1955). However, the basic assumption, that the endogenous level of auxin is supraoptimal in roots, has not yet been proved without doubt (Audus and Das, 1955). Again, it would be difficult to explain the behaviour of plagio- and diageotropic organs according to this theory

(Bennet-Clark and Ball, 1951). The mechanism of transport from the upper to the lower half of the root, as suggested by the theory, is by no means very clear. It is possible that the current from upper to lower half of root tip carries a precursor and not an auxin. In view of difficulties encountered to explain geotropic responses of roots of Pisum, Audus and Brownbridge (1957) suggested that such stimulation brings about a de novo production of an inhibitor in the underside of the elongating cells of root tips. Bennet-Clark et al. (1959) supported and confirmed this suggestion, but could not demonstrate any difference in auxin content in the control and geo-stimulated tips. That the geotropic stimulation can bring about an increased production of auxin in stems of grasses and hypocotyls of Lupinus was suggested a long time ago (Schmitz, 1933; Brian, 1942). Rufelt's (1957) observations indicated, again, that geotropic curvature is not due to endistribution of the auxin in the tissue, but due to increased production in the lower half in relation to the upper half. However, the precise role of auxins in geotropic phenomenon remains still unsolved.

It would appear from this discussion that in root growth the nature and function of endogenous growth substances are very obscure. No indisputable experimental evidence in the

light of endogenous picture of auxins has yet been presented to resolve and explain the diverse physiological problems of this field.

The present investigation was carried out with the hope of furnishing answers to at least some of the many enigmas of hormone regulated root growth.

It has not been the purpose of this introduction to review the extensive work that has been done in this field. However, elaborate treatments of the problem can be found in reviews by Burstrom (1953), Torrey (1956) and Åberg (1957).

(b) Problems related to paper partition chromatography for separation and estimation of growth substances

It is unnecessary to discuss here the spectacular advancement of auxin research since the introduction of chromatographic technique into this field. A comprehensive idea can be obtained from the relevant literature (Gordon, 1954; Natural auxins part of Wye College Symposium, 1956; Bentley, 1958; and others) and books (Leopold, 1955; Smith, 1958; Larsen, 1955; Audus, 1959). However, it would be appropriate to discuss certain technical aspects of chromatography and assay of growth substances, because the present investigation has been carried out following that technique.

1. Extraction. The great difficulty about finding a suitable method for extraction of plant growth substances is that one is never quite sure just what is being looked for, or indeed, in an active extract, what has been found. Apart from this, different solvents extract different amounts of substances or some growth factors may be extractable in one solvent but not in others. If the main aim of chromatographic investigation is to determine the actual amount of substance or substances present in the tissue, such differential solubility can present misleading picture of the auxin constitution.

If a complete extraction of growth substances is needed and no more and no less than what is present in the tissue, one has got to be careful about enzymatic and nonenzymatic formation and destruction of auxins.

Non-enzymatic. Various tissues are known to continue to release auxin over very long periods even up to several years. This process seems to be temperature insensitive (Link, Eggers and Moulton, 1941; Terpstra, 1953; Thimann and Byer, 1942) and only seems to occur in presence of water (Gustaffson, 1941; Thimann and Skoog, 1940; Wildmann and Muir, 1949). These properties plus that of continued release at low temperatures (Vlitos and Meudt, 1954) (-10°C) and after boiling (Luckwill and Woodcock, 1950) point out to a nonenzymatic process,

which is thought to be hydrolysis, probably of an auxin-protein complex. (Thimann and Byer, 1942). However, the most significant result, from a practical point of view, has been furnished by Vlitos and Meudt (1954), who found that even in low temperature alcohol extraction, TTP could be converted to IAA (and possibly IPyA) when water is present. No such conversion takes place when absolute alcohol is used. Thimann and Skoog (1946) also observed that long period release of auxin can be stopped by using dry alcohol.

Enzymatic. It has also been frequently noted that more auxin is obtained at relatively higher temperatures. It may be stopped by boiling. Tambiah (1951) could find no release after a sunflower seedling pulp had been boiled and he obtained about 90% recovery of IAA added to such a pulp. A rather more satisfactory inactivation technique would be to freeze the tissue and to carry out extraction at low temperatures. Terpstra (1953) and Wildman and Muir (1949) showed that enzymatic auxin formation ceases at about 4°C.

For the determination of free and bound auxins from tissues short (low temperature) and long (high temperature) periods of ether extraction has been employed in several cases.

Destruction of auxin can also take place non-enzymatically and enzymatically.

Non-enzymatic. Kaper's (1957) investigations have shown that stability of IPyA will depend on the pH of the medium. It is again known for a long time that IAA is stable in warm alkali but instable in warm acid (Thimann, 1935). As pigments in extracts can sensitise IAA photolysis (Jerchel and Muller, 1951), it is better to carry out extraction and other manipulations in the dark, or in diffused light. In view of the unknown nature of growth substances it is always advisable to carry out experiments in minimum light and temperature conditions.

Enzymatic. Inactivation of auxin due to enzymatic reactions can be stopped if low temperature is maintained during extraction. Alcohol extraction has the advantage that it precipitates the enzymes. Some workers have used enzyme inhibitors (di-ethyldithiocarbamate or KCN), but in view of possible interference in bioassay low temperature blocking seems to be more satisfactory.

Ether has been extensively used in extraction but objections against it are: (a) it extracts large quantities of lipoidal substances; (b) it forms a stable emulsion with water; (c) it may contain organic peroxides which interfere with bioassay and chemical assay. Water has been used as a solvent for extraction from tissues, but it is liable to freeze at temperatures suitable for enzyme inactivation.

From these considerations alcohol seems to be a suitable solvent for extraction.

2. Purification of the crude extract. It has been observed that crude extracts (i.e. after evaporation of alcohol) contain large amounts of residues which interfere with assay if directly chromatographed, so purification of extracts is necessary. There are at least three main purification methods that have been used in recent works. They are: (i) purification by fractionation (Bennet-Clark et al., 1952; Kefford, 1955); (ii) acetonitrile purification (Nitsch, 1955); (iii) purification by chromatography (Nitsch, 1955).

In the fractionation method one encounters difficulties in shaking water and ether which tend to form an emulsion in the presence of impurities. This difficulty was overcome by some workers by using a Kutscher-Steudel extractor. But this has its disadvantages too. To obtain a steady flow of ether for extraction a temperature range of 35° - 40°C (or above) has to be maintained. This heat treatment is objectionable in view of the unknown nature of all growth substances, since up to the present date we have inadequate knowledge of the heat stability of all the growth substances that occur in the plant tissue. This fractionation method does not seem to be completely efficient. It is often

observed that neutral substances like IAN leak through the acid fraction. The neutral fraction often contains considerable impurities, which make it difficult to work with. In the application of the neutral fraction on chromatographic paper, the impurities affect the course of the chromatographing. One way of overcoming this problem is by using double chromatography. Satisfactory results have also been obtained by running the extract through Kieselguhr columns (Himberg, 1958).

In acetonitrile or chromatographic method of purification the efficiency of the method will depend on the solvent used. However, all three methods have been used in the present investigation and their merits and demerits will be discussed later.

3. Chromatography. Sen and Leopold (1954) and Stowe and Thimann (1954) have furnished extensive information about the chromatography of indole compounds. Other workers have also listed Rf. values of various compounds when chromatographed under their particular conditions. Bentley (1958), on the basis of Nitsch's work, remarked that different extraction solvents and different chromatography solvents give different auxin pictures, both qualitatively and quantitatively. She has also discussed the solvents best suited for auxin investigations.

Selection of solvent for separation of plant auxins must fulfil the following conditions: (i) it must give good separation; (ii) it must not react with growth substances; (iii) it must not help the growth substances to break down during the process of chromatography; (iv) it must evaporate completely without leaving any residue.

There are again dangers of inactivation or decomposition of growth substances while chromatographing, drying and storing of chromatograms. Kefford (1955) observed that there is no advantage in developing the paper in nitrogen but considerable loss of IAA was observed on leaving the chromatogram in air and light.

Inactivation or loss of activity can also take place during elution from the paper. Audus and Thresh observed about 76% loss of IAA activity from chromatogram segments left to elute for 12 hours. They thought this might be due to bacterial decomposition in the relatively unsterile conditions used in experiments.

4. Bioassay. The most important and perhaps the most difficult part of the whole operation is bioassay. The most popular bioassay methods in chromatographic investigations are: (i) oat coleoptile assay (Kefford, 1955); (ii) oat mesocotyl assay (Nitsch, 1955); (iii) wheat coleoptile

assay (Cartwright et al., 1955); and (iv) pea root assay (Audus and Thresh, 1953). Other methods of assay (cress-root assay, lettuce germination assay, sycamore leaf-disc assay, etc.) have also been used by workers according to the requirements of their investigations.

However, the following points are important in bioassay and need careful consideration:-

1. Different assay methods may produce different quantitative and qualitative pictures of auxin present in the tissue.
2. Not all growth substances may be active in one assay method.
3. The paper might have some growth inhibitor.
4. Variations in sensitivity in assay might result from (i) biological differences of seeds or grains obtained at different times of the year; (ii) non-uniform growth of assay material due to adverse germination conditions; (iii) air pollution.

A statistical treatment of differences obtained in biological assays has been made previously by Audus and Thresh (1956) and recently by Walker et al. (1958). The results of their investigations show that the occasion variance is very much greater than that due to assay error

and is therefore highly significant. It was also observed (Walker et al., 1958) that the variance of the mean is decreased more by the number of replications (samples) than by increasing the number of section per vial.

(c) Statement of the problem and plan of attack

In view of the previous discussion on hormone regulated root growth, the present work was undertaken to study the relationships of endogenous auxins and its overall growth process. The primary objective, however, was to separate and assay the amounts of individual auxins that occur in the root tissue. Paper chromatographic separation of growth substances and their estimation by bioassay methods was thought to be adequate for present investigation. In order to picture the exact amounts and nature of growth substances that occur in roots, careful examination of purification methods has to be made. Both qualitative and quantitative differences of auxin content might be recorded in the same tissue, following different methods of extraction, purification and chromatography.

When these problems are resolved, the basic physiological problems need a critical approach. One should know first what are the growth substances that are present in the tissue. In order to know this different assay methods have to be applied to analyse the biological activities of growth substances that occur in different fractions of the root extract. It is a well-known fact that not all substances can be detected in one assay method. Again, to go further

in this problem, two-dimensional chromatography in two different solvent systems has to be attempted to separate substances occurring at same Rf. values.

Having obtained necessary information about the nature of the growth substances, a special emphasis has to be attached to the study of qualitative and quantitative changes of growth substances in the whole root system, as it increases in age. The results should verify the concepts of ageing of roots, as presented by Pilet (1951). Moreover, it should furnish information about exact amounts of growth substances that occur in different stages of root growth. On the basis of this and corresponding growth data, useful information may be obtained about the hormone regulated root growth.

In order to go deeper into the problem, a study of the distribution of growth substances in different parts of the root, at its different ages, should be more profitable. We have very little data on this problem and correlation of growth phenomenon in roots would be impossible if the pictures of auxins in different parts of roots at different ages of plants are not clearly defined.

The problems related to hormonal relationship of laterals and the main root can again be attacked by estimation of

growth active compounds in the lateral tips, in presence and absence of the tap root tips. The basis objective, however, would be to establish a direct relationship of lateral growth and its endogenous balance of auxins. Although considerable information has been collected in relation to lateral initiation, the quantities and natures of auxins that occur in the tissue have not been properly studied.

A direct and straightforward attack on problems related to geotropism would be to analyse the growth substances that occur in the upper and lower halves of the root tip. Although it has been done before (Hawker, 1932), chromatographic analysis of the extracts is needed to verify the previous observations. The theory of geotropism extended by Audus and Brownbridge (1957) can again be tested by assay of auxins in the geotropically stimulated root tips. If a de novo production of inhibitor is involved in the process, it can be traced, by assay of auxins from root apex materials which are subjected to geotropic stimulation for different lengths of time. Again, from estimation of free and bound auxins from control and stimulated root apex material, it can be deduced whether a de novo production, rather than a liberation of bound auxin, is involved in the process.

The reports of water soluble auxin-precursors that occur in roots need further verification. Spontaneous break-down and interconversion of these substances has added new complexities to problems of root growth. Nevertheless, their biological activities towards different assay methods should furnish some information about their mode of action in different tissues.

C H A P T E R I I.

MATERIAL AND METHODS

Material:

(a) Source of material for extraction purpose

Peas (Pisum sativum var. Meteor) and broad-beans (Vicia faba var. Green Leviathan) have been used for the purpose of the present investigation. They were obtained through Carters Tested Seeds Ltd.

Peas or broad beans were soaked for 24 hours in aerated running water in the washer devised by Audus (1956). They were allowed to germinate in sieved, sterilised and damped sand in earthern pots (diameter approximately 9 inches and depth 4 inches) with a single drainage hole in the centre. This hole was covered with a watch-glass before filling with sand. Seeds were evenly spaced with their radicals pointing downwards. Aluminium trays were kept underneath the pots and tops were covered with clean glass plates or aluminium trays. They were kept in a dark cupboard at 25±1°C.

After 2 to 3 days growth the seedlings showing uniform growth were transferred to water-culture tanks. The roots were threaded through small diameter holes in black perspex sheets supported at the water-surface in glazed earthenware

tanks. Each perspex sheet was shaped accurately to fit the tanks. Aeration was by a slow stream of bubbles coming through a carbon filtered air source. Water in the tank was changed on every 4th day. After a suitable interval of time the sheets were removed from tanks and the roots were collected for extraction.

(b) Source of material for bioassay purpose

Four bioassay methods were employed in the course of the work - oat mesocotyl straight growth assay, oat coleoptile straight growth assay, wheat coleoptile straight growth assay and pea root section assay. Of these the first and the fourth method have been extensively used for the purpose of this present investigation.

Avena sativa (var. Victory) or wheat (var. Eclipse) seeds were soaked for 2-3 hours in tap-water. The soaked seeds were laid down on moist (sand : water - 4 : 1) sterilised sand in glass half bricks and the seeds were covered up with a thin layer of moist sand. Another half-brick was kept above it to reduce evaporation. The seedlings were grown in complete darkness for 72 hours at 25±1°C. In the case of Avena coleoptile assay, the plants were taken out of the dark cupboard and were exposed to light for about three minutes 16 hours or so before they were required. This had the effect

of suppressing mesocotyl elongation, and in comparison with seedlings grown in continuous darkness, promoted coleoptile growth.

The peas were grown in sand and in darkness as has been described before. For growing peas for bioassay purpose special care was taken in planting them with radicals pointing downwards, otherwise curved roots were produced and could not be used for section cutting.

Methods:

(c) Extraction technique

Roots collected as described before were immediately weighed and deep-frozen at -14°C. The frozen tissue was then macerated with minimum volume of chilled absolute ethanol in a "Magimix" (about 4 times the weight of the tissue) and the brei was left in darkness at -14°C. for 20 hours. The extract was then filtered under suction.

For extraction of auxins from root tips, the tips were cut and immersed immediately in chilled alcohol and kept in a flask in a waterbath filled with iced water. The weight of the tissue was determined by subtracting the weight of flask plus alcohol from the weight of flask, alcohol and tissue. This was followed by low temperature extraction for 20 hours in darkness. At the end of this period the extract was filtered and the filtrate was collected.

In most cases purification of the extract and subsequent bioassay was done immediately after extraction. In some cases when the extract had to be stored, it was kept in the deep-freeze at -14°C in darkness.

(d) Purification methods

(i) Acetonitrile method

In the initial stages of this investigation attempts were made to purify the extracts by this method forwarded by Nitsch (1955, 1956).

The ethanolic extract was evaporated down under reduced pressure to very near dryness. The resulting dark brown syrup was extracted with 10ml. of acetonitrile, followed by 4 washings with 5 mls. of acetonitrile each time. The washings were combined and partitioned against hexane 3 times, using 15 mls. of hexane each time. The hexane was discarded and acetonitrile was again concentrated under reduced pressure. The residue was taken up in ether (Analar grade) for application to the chromatogram strip. At no step of these operations the temperature went above 28-30°C.

(ii) Water chromatography

Here also alcohol was evaporated off under reduced pressure. The gummy residue with water was then directly streaked on the starting line of a 15 cm. x 46 cm. Whatman

No.2 paper as a band, with the help of a glass jet. It was found that such a method of loading the chromatogram takes a very long time, and the whole operation is extremely difficult. When the gummy band on the paper was dry it was run in distilled water. Though the movement of water under ordinary conditions was fairly fast, it travelled at an extremely slow rate due to the presence of the gum in the starting line. After developing and drying the paper, the starting line with the fatty substances was cut off, and the rest of the paper was cut into small pieces and was eluted with alcohol. A number of solvents were tried, to find out the suitable solvent for elution. The results will be described later.

It was found that $3\frac{1}{2}$ hours is the optimum time for elution with alcohol. So paper pieces were eluted with alcohol for $3\frac{1}{2}$-4 hours on a mechanical shaker. The alcohol was then filtered off and brought down to a small volume for application on paper.

(iii) Fractionation method

This is primarily based on the method used by Kefford (1955) and Audus and Thresh (1956), with slight modification.

Alcohol was evaporated off under reduced pressure. In the later stages of this work reduced pressure distillation

was done with the help of a flash-evaporator. The aqueous residue was acidified to pH3 by N/10 sulphuric acid and shaken with aliquot amounts of ether. Ether was changed four times and each shaking was continued for 15 mins. In the case of emulsion formation the solution was deep frozen and the ether layer was separated out. The ether fractions were combined and further shaken with aliquot amounts of 5% $NaHCO_3$. Here also bicarbonate solution was changed 4 times and each shaking was continued for 15 mins. The bicarbonate fractions were combined and the ether part was also stored for chromatography. The bicarbonate solution was then brought down to pH3 by the addition of phosphoric acid (sp. gr. 1.75) and was partitioned 4 times against ether and the ether fractions were combined and the bicarbonate solution was discarded. This acid ether fraction was dried overnight over sodium sulphate and brought down to a small volume under reduced pressure for application on paper. The neutral ether fraction was also similarly treated.

In order to analyse the water soluble (ether insoluble) fraction the following procedure was followed: the aqueous fraction left over after shaking with ether at pH3 was neutralised with barium hydroxide and filtered and concentrated under reduced pressure in a weighed flask. Special care was

needed at this stage to prevent frothing. The weight of dark brown residue was determined and taken up again in a known volume of water.

A number of solvents were tried to extract the growth active compounds from this syrup - the results of which will be described later. The final method was as follows: a small amount of the gum (of known tissue weight) was extracted overnight with 3 ml. of alcohol at 2°C, in darkness. The alcohol was poured off, centrifuged and brought down to a small volume under reduced pressure for application on paper.

(e) Chromatography

The final purified and concentrated extract was spotted 10 cms. from one end of a 2 by 50 cms. (for oat coleoptile, oat mesocotyl, and wheat coleoptile) or 3.8 by 50 cms. (for pea root assay) Whatman No.2 chromatographic paper. A stream of nitrogen or air was applied on the spot to facilitate drying. The ascending and descending methods were used, and the marker chromatograms of synthetic IAA or IAA + IAN developed concurrently. The solvent used in this investigation was iso-butanol : methanol : water :: 80 : 5 : 15 (after Nitsch, 1956). The pH of the solvent was found to be neutral.

The descending chromatograms were developed in glass tanks and were further enclosed in a larger glass tank. The

larger tank with two smaller tanks was again covered up by a light-proof wooden cover. The chromatographic tanks were kept in a windowless fully enclosed room of a centrally heated building and the temperature remained more or less constant. But at initial stages of this work alarming differences in Rf values were observed between two tanks standing side by side with identical solvent and paper. Previous rubber fittings of the tanks were changed and all-glass apparatus was fitted up. This resulted in a further shift in the Rf. value of IAA. Earlier Rf. was about 0.25, which agreed closely with that found by Nitsch (1956); later, with glass fittings, the value was stable around 0.55.

Descending - The paper strips were suspended in the tanks between glass clips with their upper ends dipping into a glass trough and were left to equilibrate for 1 hour with the vapour of the particular solvent in the bottom of the tank. Then a measured amount of solvent was poured into the trough through a small hole in the glass lid of the tank and allowed to run down the strip for approximately 14-16 hrs. in the dark. During this time the solvent front travelled a distance of about 24-28 cms.

Ascending - In a few experiments ascending chromatograms were run. These were carried out in 500 ml. graduated

cylinders with sealed tops and a paper strip suspended by a thread on a moveable glass tube.

Chromatograms were dried in still air and assayed immediately. In cases when chromatograms had to be stored they were kept in a vacuum-desiccator at - 14°C.

(f) Bioassay techniques

Oat and wheat coleoptile extension test, oat mesocotyl extension test and pea root extension test have been applied in this investigation for determination of activities of growth substances obtained from plant extracts. Of these, Oat mesocotyl and pea root assay were used for quantitative estimation of activities with reference to standard IAA calibration curves.

(i) Oat mesocotyl section extension test

This assay method has been most frequently used in the present investigation.

The general method is based on that of Nitsch (1955). The cutter consisted of two razor blades fixed firmly on a steel holder with a perspex bridge in between them. The cutting edges of blades removed sections 3.14 mm. in length. The other part of the cutting equipment was an accurate perspex guide (Fig. 1.a.) used for positioning the blades which had two grooved sides, one of which was 2.0 mm. and the other 3.0 mm. (~~Fig. 1.b.~~)

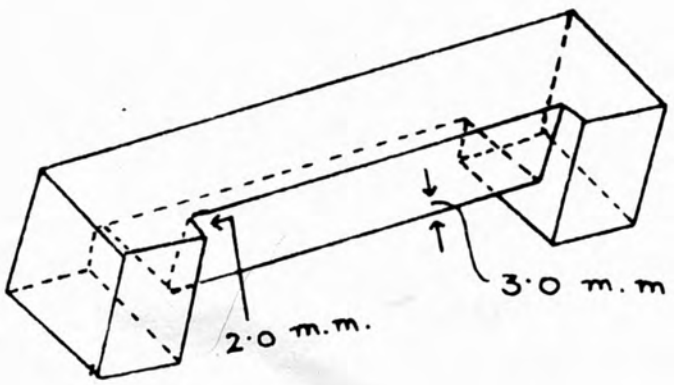
Fig.1. a. Perspex guide for cutting coleoptile and mesocotyl sections.

b. Method of cutting oat mesocotyl sections.

c. Method of cutting oat and wheat coleoptile sections.

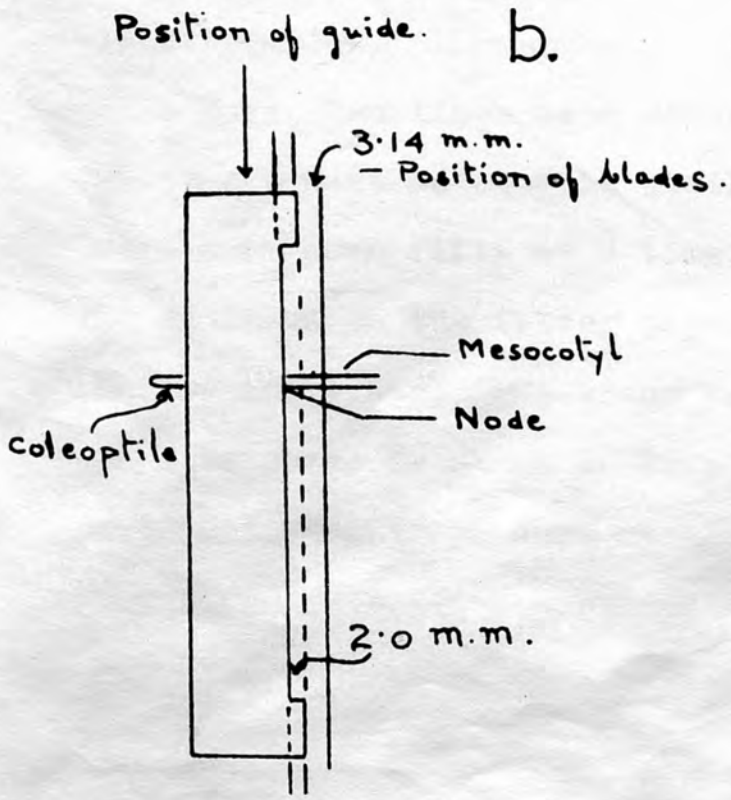
FIG.1.

Perspex guide



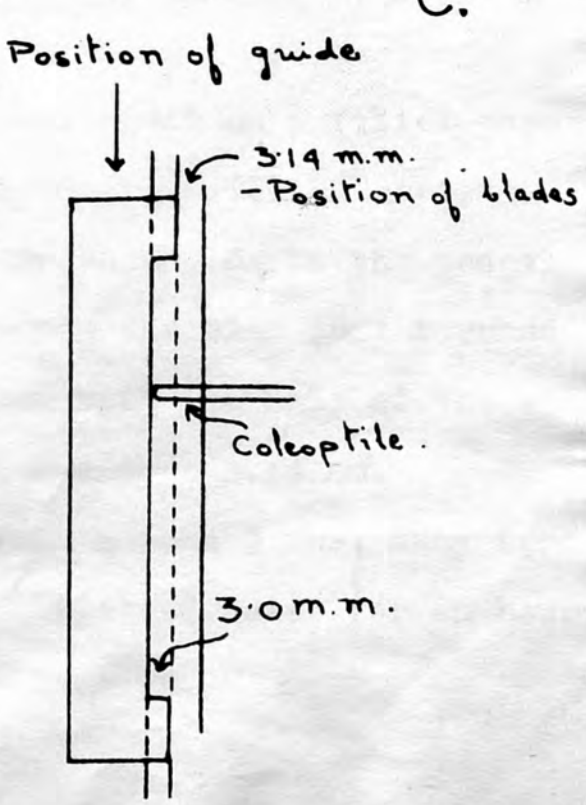
a.

Mesocotyl



b.

Coleoptile



c.

At first two pencil lines 2 mm. apart were drawn on a filter paper and made moist with distilled water. The coleoptile with elongated mesocotyls were harvested and laid down at right angles to the lines, with nodal zones arranged along one of the lines, straddled by the guide and the cutter brought down into position as shown in Fig. 16. Thus 3.14 mm. sections were obtained from a zone 2 mm. away from the node.

This system enabled 50 sections to be cut in one stroke and allowed a cutting rate of about 250-300 per hour. All manipulations were done under a 'Kodak' safe lamp with a yellow-green filter. Sections were soaked for 1 hour on a muslin stretched over distilled water in a perspex box.

(ii) Oat and wheat coleoptile assay section extension test

These assays have been occasionally used in the present investigation.

Here, two lines were drawn 3 mms. apart on a filter paper which was made moist with distilled water. The coleoptiles were laid down fifty at a time, at right angles to the pencil lines drawn on the filter paper so that the tips just touched one of the lines. Sections were cut with the help of the perspex guide as shown in Fig. 1.c. and thus 3.14 mm. coleoptile sections were obtained from a zone 3 mms. away from the tip. Sections were soaked in distilled water for an hour before distribution in vials.

All manipulations were done under a 'Kodak' safe lamp with a yellow-green filter. Coleoptile sections could be cut at a slightly faster rate than mesocotyl sections.

(iii) Pea root section extension test

Straight roots from 3 days peas were used for sectioning. A number of experiments were performed to improve the bioassay method devised by Audus and Thresh (1953). The results will be described later. For the chromatographic investigation 2 mm. sections were used, taken from 1 mm. away from the root tip of a 3 days' old plant. Seedlings were washed to clear the sand and sections were cut with a special guillotine (Audus and Gerrard, 1953) and placed in a Petridish containing distilled water. 100 sections were cut at a time, randomised and distributed in bioassay vials.

All manipulations were done under diffused laboratory light.

(g) Conditions of section growth, measurement and presentation of results

In all bioassay methods special care was taken so that sections in each vial were subjected to equal lengths of time at the time of measurement.

Bioassay was carried out in a small cylindrical glass vessel 1.0 inch in diameter and 1.0 inch high. In the case

of oat and wheat coleoptile assay and oat mesocotyl assay 1.0 ml. of bioassay medium has been used. For elution from paper or for IAA calibration tests the bioassay medium consisted of Potassium monohydrogen phosphate (10^{-2}M), citric acid ($0.5.10^{-2}$M) and 2% (medium buffered at pH 5.0) of sucrose. The dried paper chromatogram was divided up into 20 equal parts (for fineness of resolution), with the help of a standard Rf-value key previously drawn out on a board. Each strip was cut into fine segments before putting in vials, and then buffered medium was added in each tube. The tubes were numbered and stoppered with corks having a hole in the middle and filled in with loose cotton-wool plugs. Usually 10 sections (occasionally 8) were placed in each vial and were free-floating in the solution.

For pea root assay similar procedures were followed excepting the following changes:

a. 0.5% (0.75 ml.) of sucrose solution was used for elution and IAA calibration.

b. Paper segments from chromatograms were wrapped around small squares of glass (1.5 x 1.5 cm.) cut from a microscope slide and inserted into the glass vessel (according to the method of Audus and Thresh, 1953).

c. Sections were placed on the wet paper in the vial and they were more or less stationary.

For each assay 10 vials with 10 sections (occasionally 8) in each were kept as controls. Solvent-run chromatographic paper taken from regions above the starting point was used for controls. For all the assay methods a growth period of approximately 18 hours was allowed and throughout this time the vials were gently rocked on an electric shaker in darkness at a constant temperature of $25 \pm 1^{\circ}C$.

Growth was recorded photographically by laying out the sections from one tube in a row on a glass plate. Several such rows were arranged according to the size of the photographic paper and the glass plate was placed on the negative carrier of an enlarger. Shadow-graphs were recorded at a magnification of times four. The method is very quick and eliminates all personal error. The total lengths of all sections from each tube were measured to the nearest millimetres from the enlarged images and growth was expressed as % of the growth of control sections, i.e.

$$\frac{\text{total section growth in assay vial}}{\text{Mean of total section growths in control vials}} \times 100.$$

It has been previously stated that ten control tubes were included in each experiment containing sections in pure bioassay medium. Growth of sections in each vial was determined and the average of these was taken as the control level of growth. Significance of results was attested by

determining the standard deviation of the results from the ten control tubes, according to the formula

$$S.D. = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$$

where $\bar{x}$ = grand mean for all ten tubes.

x = mean growth of sections in each tube.

n = total number of control tubes.

Fiducial limits, i.e. twice the standard deviation expressed as per cent of $\bar{x}$, were taken as the limits of significance. Mean section lengths differing from $\bar{x}$ by greater than twice the standard deviation are significant at the 0.05 probability level.

In a few experiments length measurements were done with the help of a travelling microscope. Fiducial limits calculated from standard deviations of 10 control results were taken as the criterion of significant deviation, indicative of active spots on chromatograms. On histograms, obtained from chromatographic analysis of root extracts, these significant deviations are marked with black dots.

(h) Methods of tissue feeding with synthetic hormones and precursors

Roots of 5-6 days' old broad beans have been used in these experiments. The roots were harvested, randomised and

cut into 2.5 - 3 cm. pieces. 10 gms. of tissue were floated in 35 mls. of buffered (PH5) 2% sugar solution in 250 ml. flasks. Each ml. of buffer contained 5 mgs. of DL-TTP, or 1 mg. of IAN, or 1 mg. of TNH2, or 1 mg. of IAA. The root tissues were incubated with these compounds for 20 hours on a shaker in darkness at 25±1°C. After incubation the flasks were placed in boiling water bath for 10 minutes and then cooled. The contents and the wash-water from flasks were placed in a mortar and triturated. The resulting brei was centrifuged and the supernatant was collected. The solution was separated into acid ether, neutral ether and water soluble fractions according to conventional method. The water soluble fraction was lyphilized and directly spotted on paper.

(i) Methods of extraction of free and bound acid auxins from root tips

(1) Free auxin. Root tips were harvested and put directly in cold ether. Extraction was continued for 3 hours at +2°C. The ether was decanted off and the root tips were further washed twice with fresh ether and all the washings were combined. This ether was then partitioned against sodium bicarbonate (5%) solution and the bicarbonate solution was again partitioned with fresh ether at PH3, following the standard method previously described. Final acid ether

fraction was brought down to a small volume and under reduced pressure and applied directly to a chromatogram.

(2) Bound auxin. After extraction of free auxin, the same tissues were left with fresh ether for 48 hours at +28°C. The ether was decanted off and the washings were combined with it. The acid ether fraction was obtained following similar methods used for free auxins.

(j) Spraying reagents

(i) Ehrlich's reagent. 2 gms. of p-dimethylaminobenzaldehyde in a mixture of 20 ml. Hcl (sp.gr. 1.18) and 80 ml. ethanol.

(ii) Salkowski reagent. A mixture of 1 ml. of 0.5 M. $FeCl_3$ plus 50 ml. of 35 per cent (W/V) $HClO_4$.

(iii) Nitrous-nitric acid. 1 gm. of KNO_3 dissolved in 20 ml. HNO_3 (sp.gr. 1.42) was diluted to 200 ml.

(iv) 5% potassium-dichromate solution.

C H A P T E R I I I.

RESULTS

1. Preliminary investigations and development of methods.

(a) Growth conditions of pea root sections for maximum sensitivity

The assay methods of growth substances from paper need a high degree of sensitivity because only small amounts of active substances are likely to be present in plant extracts. Large volumes of growth medium cannot be used because severe dilution of growth factors would occur reducing the chances of detection and estimation of these substances. Apart from this one must consider where to cut the sections and how to grow them to obtain maximum sensitivity of the assay material. Audus and Thresh (1953) devised an assay method with pea root sections for estimating growth substances from paper. In the preliminary experiments attempts were made to study the optimum growth conditions of pea root sections with an idea to improve the method described by previous authors.

In the first experiment 1.6 mm. pea root sections (taken 1 mm. from tip) were grown in different volumes of .5% sucrose solution for 18 hours in presence and in absence of chromatographic paper (2 x 2 cm. segment, cut into small

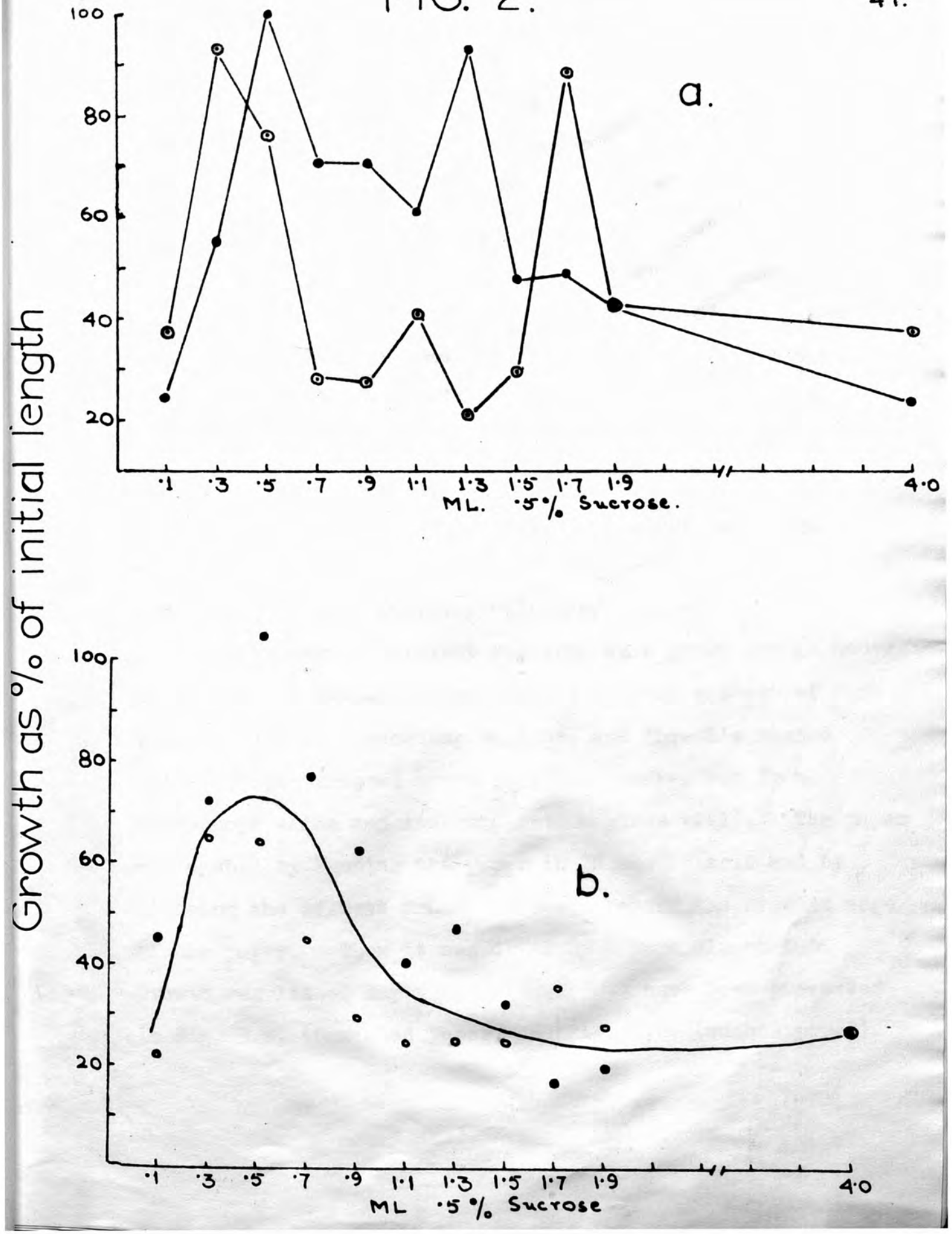
Fig.2. Relationship between the total growth of root sections (growth as percentage of initial length) over 18 hours and the quantity of sucrose medium in the growth vessel.

a. sections growing with chromatographic paper.

b. without paper.

(For explanation see text)

FIG. 2.


Growth as % of initial length
100
80
60
40
20
a.
·1 ·3 ·5 ·7 ·9 1·1 1·3 1·5 1·7 1·9 4·0
ML. ·5% Sucrose.
b.
ML ·5% Sucrose

pieces). From Fig. 2.b. it may be seen that the growth of sections even in absence of paper was very poor - the maximum growth of about 70% was obtained with 0.5 ml. of solution. In presence of paper the growth of sections was alarmingly irregular (Fig. 2.a.). In another experiment root sections were grown in 1 ml. of medium of different concentrations of IAA and 0.5% sucrose solution (plus paper strips). Growth measurements after 18 hours showed a general inhibition of sections in all concentrations of IAA and also in the control.

This irregularity in growth was thought to be due to either (i) presence of inhibitor in paper, (ii) limiting aeration, or (iii) limiting volume of medium.

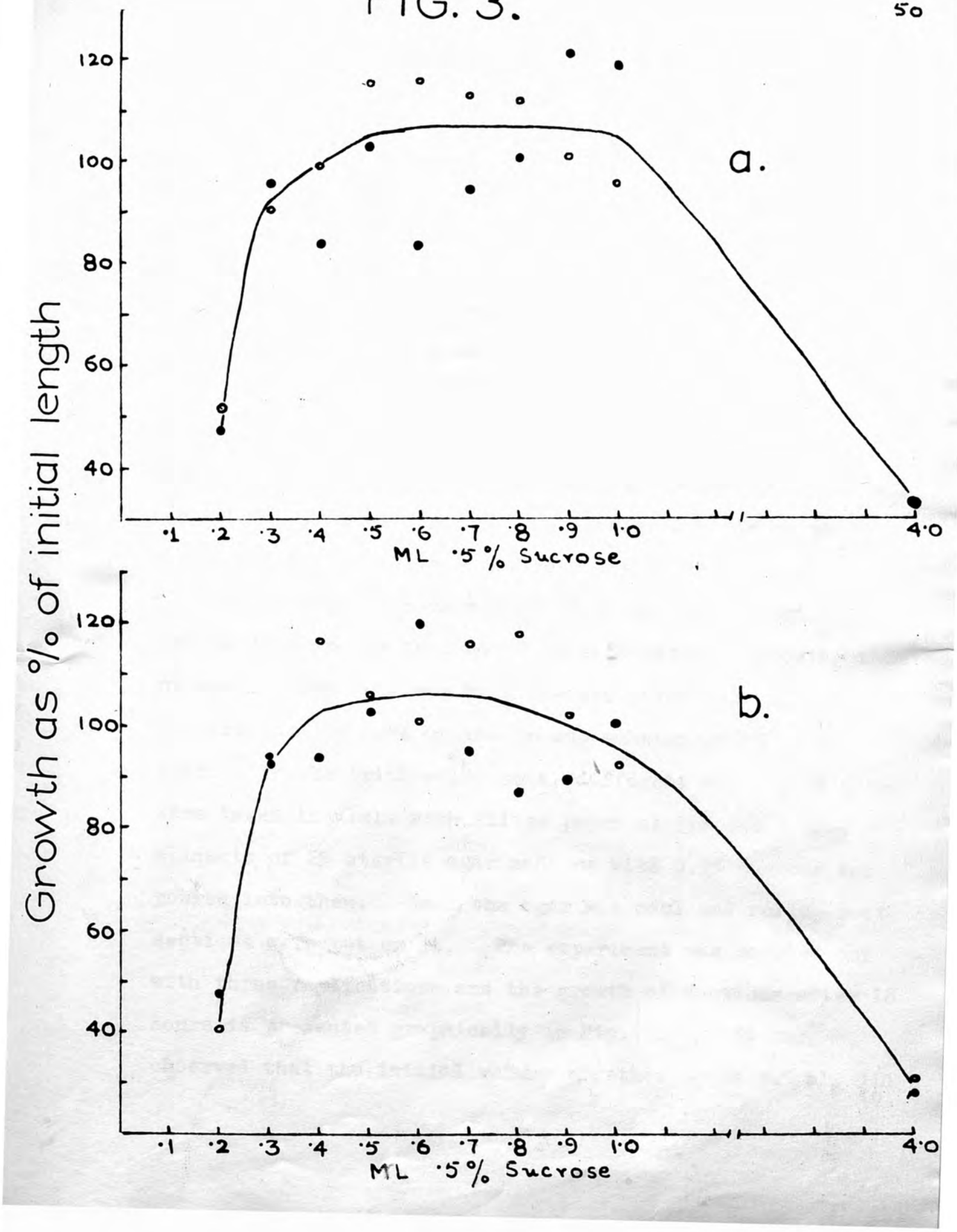
In the next experiment sections were grown for 18 hours on washed and unwashed paper with different amounts of 0.5% sucrose solution according to Audus and Thresh's method (filter paper wrapped around a glass square, cut from microscope slide and inserted into a glass vial). The paper was washed by running the paper in 2N acetic acid and by allowing the solvent front to travel beyond the distant edge of the paper. Then it was dried by a warm air stream. Growth results of duplicate experiments have been presented in Fig. 3.a. (unwashed paper) and Fig. 3.b. (washed paper).

Fig.3. Relationship between the total growth of root sections (growth as percentage of initial length) over 18 hours and quantity of sucrose medium in the growth vessel. Results obtained following Audus and Thresh's (1953) method.

a. sections grown on unwashed chromatographic paper.

b. sections grown on washed chromatographic paper.

FIG. 3.


Growth as % of initial length
120
100
80
60
40
a.
·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 4·0
ML ·5% Sucrose
b.
ML ·5% Sucrose

The growth curves in both experiments were almost identical. It was naturally concluded from these observations that growth of sections was not affected by any inhibitor on paper. It was further noted that a maximum growth of sections was just above the 100% level and this rate was more or less constant from 0.5 to 0.9 ml. of solution. Beyond that, growth of sections falls due to water-logging and lack of aeration. In contrast, when the sections were floated in larger volumes of sugar solution (10 ml. or more) and aeration was assured by shaking the flask or Petridish, growth of sections was about 200% (Fig. 4.a. in 50 ml. flask and Fig. 4.b. in 9 cm. Petridish).

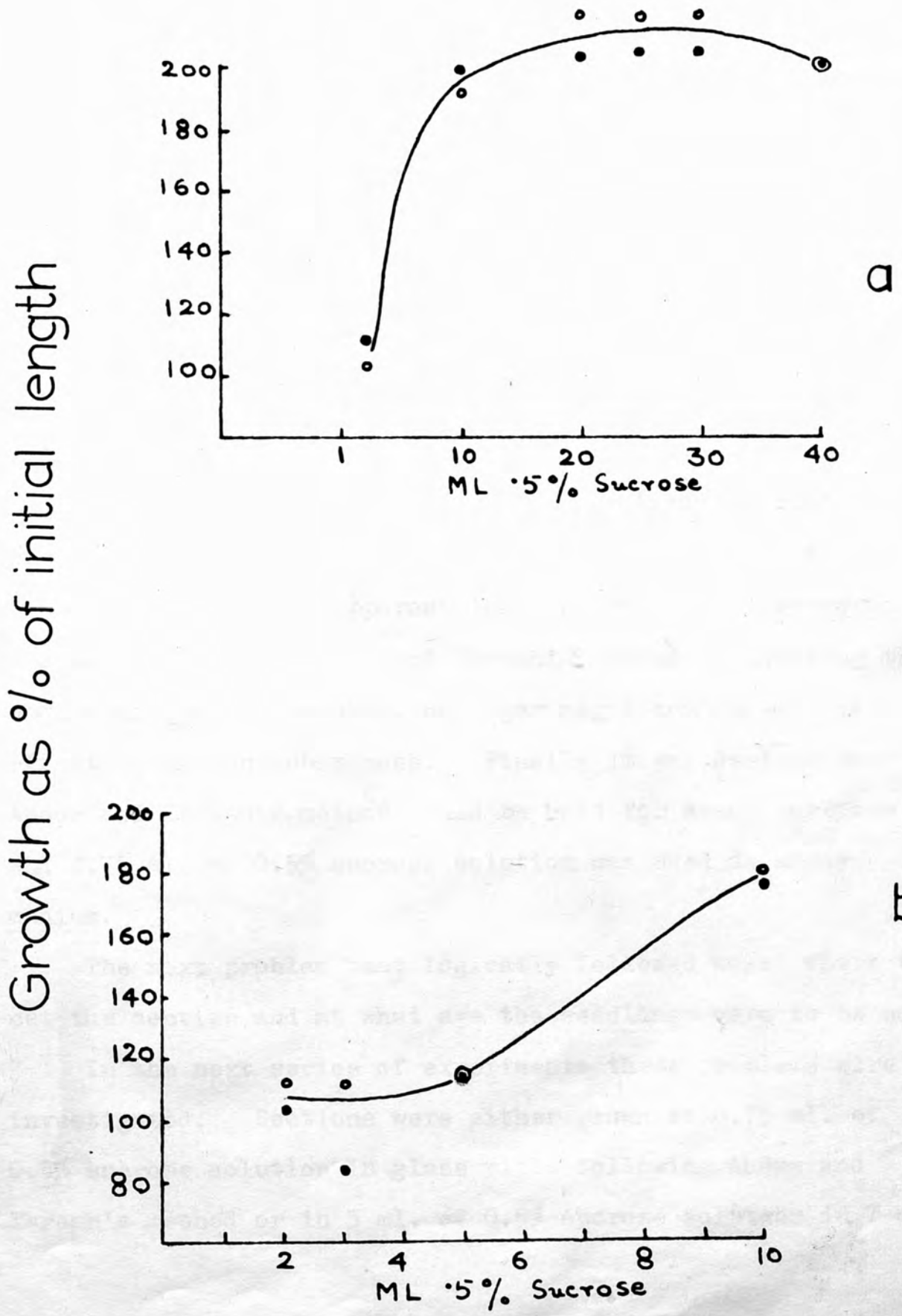
It was again thought that if aeration was limiting for section growth that could be eliminated by growing them on agar. The idea was to elute the chromatographic paper in ether and to take up the growth substances again in agar. In the trial experiment, different volumes of ether were taken in vials with filter paper strips and 1 ml. aliquots of 2% sterile agar made up with 0.5% sucrose was poured into them. When the agar was cool and solid, root sections were put on it. The experiment was carried out with three replications and the growth of sections after 18 hours is presented graphically in Fig. 5.a. It was observed that the initial volume of ether up to 1.5 ml. did

Fig.4. Relationship between growth of root sections over 18 hours and quantities of sucrose medium in the growth vessel.

a. sections grown in 50 ml. flask with different volumes of medium.

b. sections grown in 9 cm. Petridish with different volumes of medium.

FIG. 4.


Growth as % of initial length
200
180
160
140
120
100
1
10
20
30
40
ML .5% Sucrose
a
200
180
160
140
120
100
80
2
4
6
8
10
ML .5% Sucrose
b

not affect the growth of root sections but slight inhibition occurred when 2 ml. was used; but growth in all cases was below the 100% level.

In another experiment different amounts of alcoholic IAA solution were spotted on 2 x 2 cm. filter paper strips with a micropipette. They were dried in still air, cut into small pieces and eluted with 1.5 ml. ether in glass vials. Aliquot amounts (1 ml.) of sterile agar (made up with .5% sucrose) were poured into each vial and allowed to solidify. Final concentrations of IAA in the vials were: 100, 10, 1, 0.1, etc. µgm/ml. The growth of pea root sections under these conditions has been presented in Fig. 5.b.

However, it was apparent that no definite improvement has been made over Audus and Thresh's method by adopting this new technique. Moreover, hot agar might have some adverse effect on growth substances. Finally it was decided that Audus and Thresh's method could be used for assay purposes and 0.75 ml. of 0.5% sucrose solution was used as assay medium.

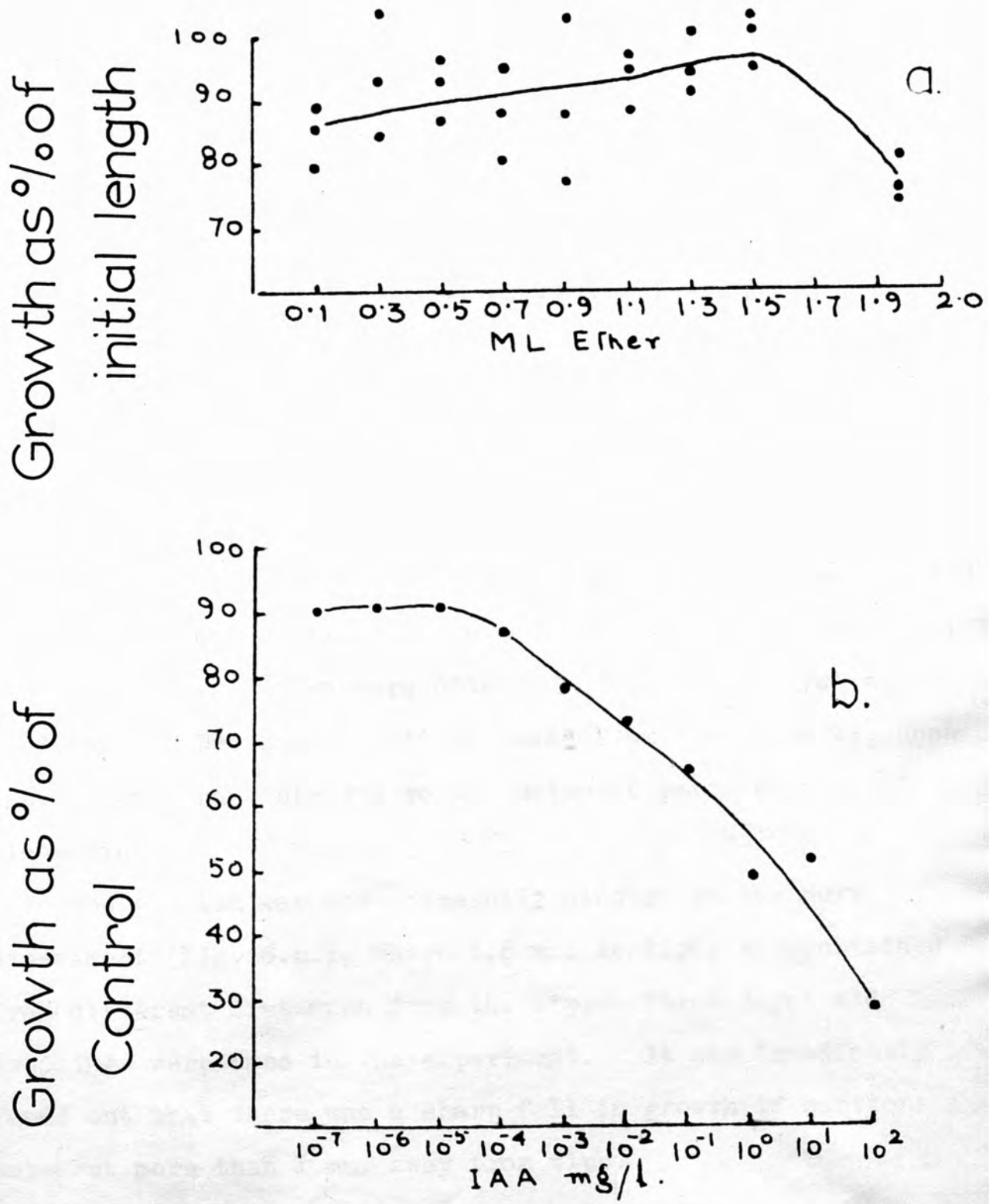
The next problem that logically followed was: where to cut the section and at what age the seedlings were to be used.

In the next series of experiments these problems were investigated. Sections were either grown in 0.75 ml. of 0.5% sucrose solution in glass vials following Audus and Thresh's method or in 5 ml. of 0.5% sucrose solution in 7 cm.

Fig.5. a. Relationship between initial amount of ether in growth vessel and subsequent growth of root sections in agar.

b. Growth of root sections on agar with different concentrations of IAA. IAA eluted from paper with ether and taken up in agar.

FIG. 5.


Growth as % of initial length
100
90
80
70
a.
0·1 0·3 0·5 0·7 0·9 1·1 1·3 1·5 1·7 1·9 2·0
ML Ether
Growth as % of Control
100
90
80
70
60
50
40
30
20
b.
10⁻⁷ 10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁰ 10¹ 10²
IAA mg/l.

Petridishes with filter paper strips (2 x 2 cm.). Ten sections were put in each vial or Petridish and three replications were kept for each treatment.

In the first experiment of this series three different types of sections (viz. (i) 1.6 mm. sections taken 1 mm. from tip, (ii) 2 mm. sections taken 1 mm. from tip, and (iii) 2 mm. sections taken 2 mm. from tip) were obtained from pea seedlings of different ages and their growths were recorded after 18 hours. From the results (Figs. 6.a. and b.) it was noted that there was always poor growth in sections obtained from 4 or 5 days' old seedlings. In 0.75 ml. medium, 1.6 mm. or 2 mm. sections taken 1 mm. from tip show almost similar growth if sections were obtained from 2 or 3 days' old seedlings. But 2 mm. sections taken 2 mm. from the tip show less growth, possibly due to exclusion of parts of zone of elongation.

The problem was more carefully studied in the next experiment (Fig. 6.c.), where 1.6 mm. sections were obtained from different distances from the tip. Three days' old seedlings were used in the experiment. It was immediately found out that there was a sharp fall in growth if sections were cut more than 1 mm. away from tips.

The next experiment was performed to investigate the effect of initial length of sections on the subsequent growth.

Fig.6. a. Effect of age of the pea seedling on the growth of root sections (1.6 mm. section taken 1.0 mm. from apex, 2.0 mm. sections taken 1.0 mm. from apex, and 2.0 mm. sections taken 2.0 mm. from apex) in 0.75 ml. of sucrose (0.5%) medium.

b. Similar sections as in (a), grown in 5.0 ml. medium.

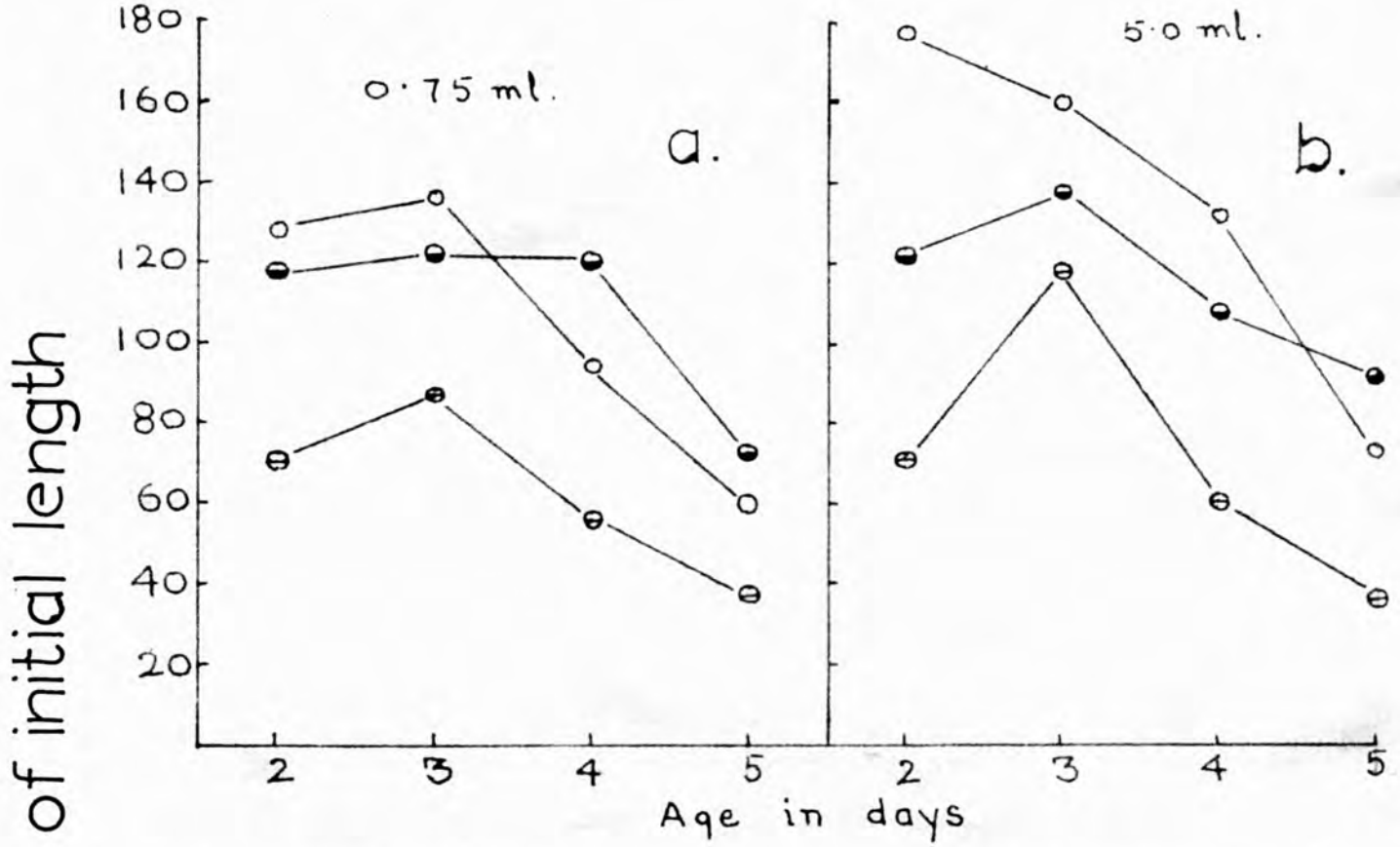
c. Growth of 1.6 mm. sections obtained from different distances from the apex.

d. Effect of initial length on the growth of root sections.

FIG. 6.

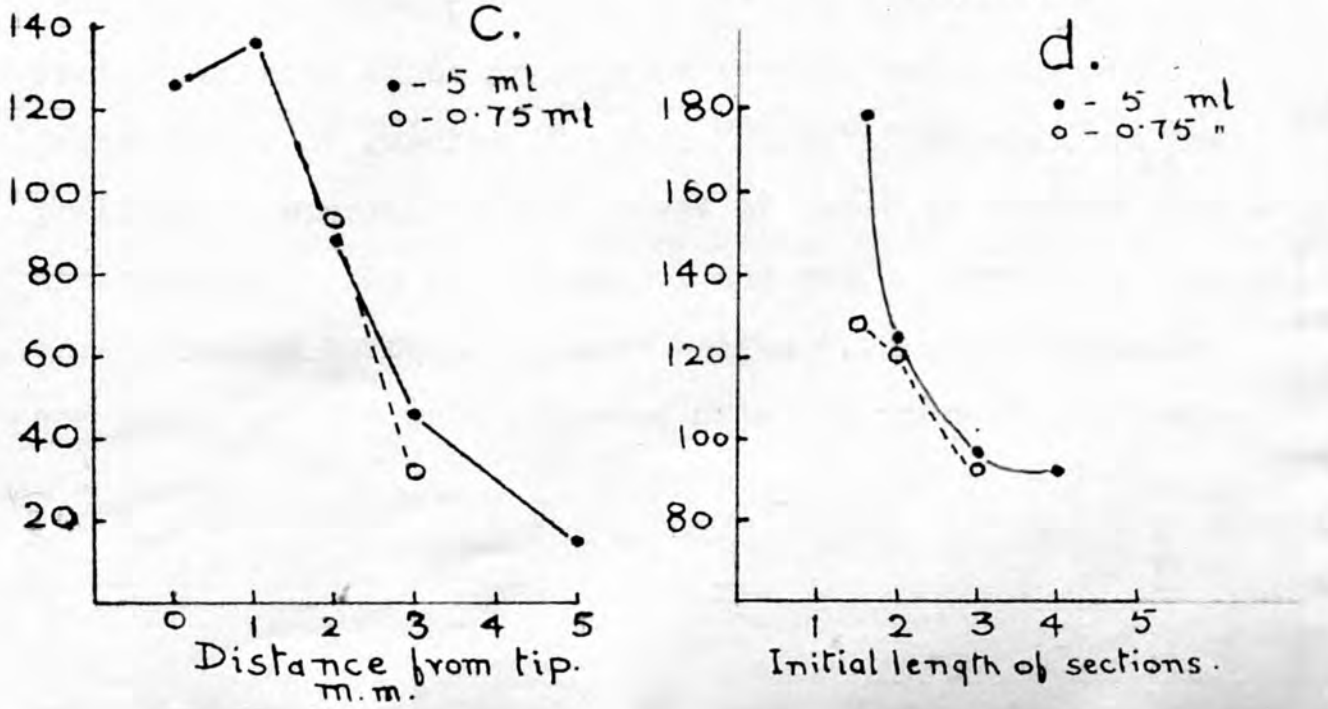
Effect of age.

o 1·6 mm section taken 1·0 mm from tip.
● 2·0 " " " " " " "
⊖ 2·0 " " " 2·0 " " "



Effect of location of sections.

Effect of initial length of sections.



Sections of different lengths were obtained from regions 1 mm. away from the tips. It appeared from results (Fig. 6.d.) that longer sections (3 or 4 mms.) show less growth; but in 0.75 ml. medium 1.5 and 2 mm. sections showed almost equal growth.

On the basis of these findings 2 mm. sections taken 1 mm. from tip were used in 0.75 ml. growth medium (0.5% sucrose solution) in later experiments.

(b) Growth conditions of oat coleoptile and oat mesocotyl sections

Investigations on this aspect have been extensively carried out by Nitsch (1955), Nitsch and Nitsch (1956) and Bentley (1950).

The assay methods (Oat coleoptile and first internode of oat) used in this investigation is primarily based on information obtained from previous authors. The growth conditions are similar to those already described in the section on conditions of section growth, measurement and presentation of results (Chapter III.e.) However, in the preliminary experiment the effect of paper on section growth was studied. The oat mesocotyl and oat coleoptile sections were grown in buffered sucrose medium with paper segments and their growths were recorded after 18 hours following

standard methods.

TABLE I.

Nature of sections	Section length (after 18 hours) as % of initial length	
	Cont. (- paper)	+ paper
Mesocotyl	124.5 ± 1.8	124.8 ± 1.2
	125.7 ± 2.2	126.2 ± 2.4
	124.8 ± 1.8	121.3 ± 3.5
	122.0 ± 1.4	124.1 ± 2.1
Coleoptile	179.1 ± 1.2	151.2 ± 1.4
	156.8 ± 1.3	151.2 ± 1.04
	156.05± 1.8	148.08± 2.4
	163.2 ± 1.9	153.6 ± 2.1
	150.4 ± 1.7	147.2 ± 1.8

The observations go in pairs and standard deviation of sections are presented against each mean.

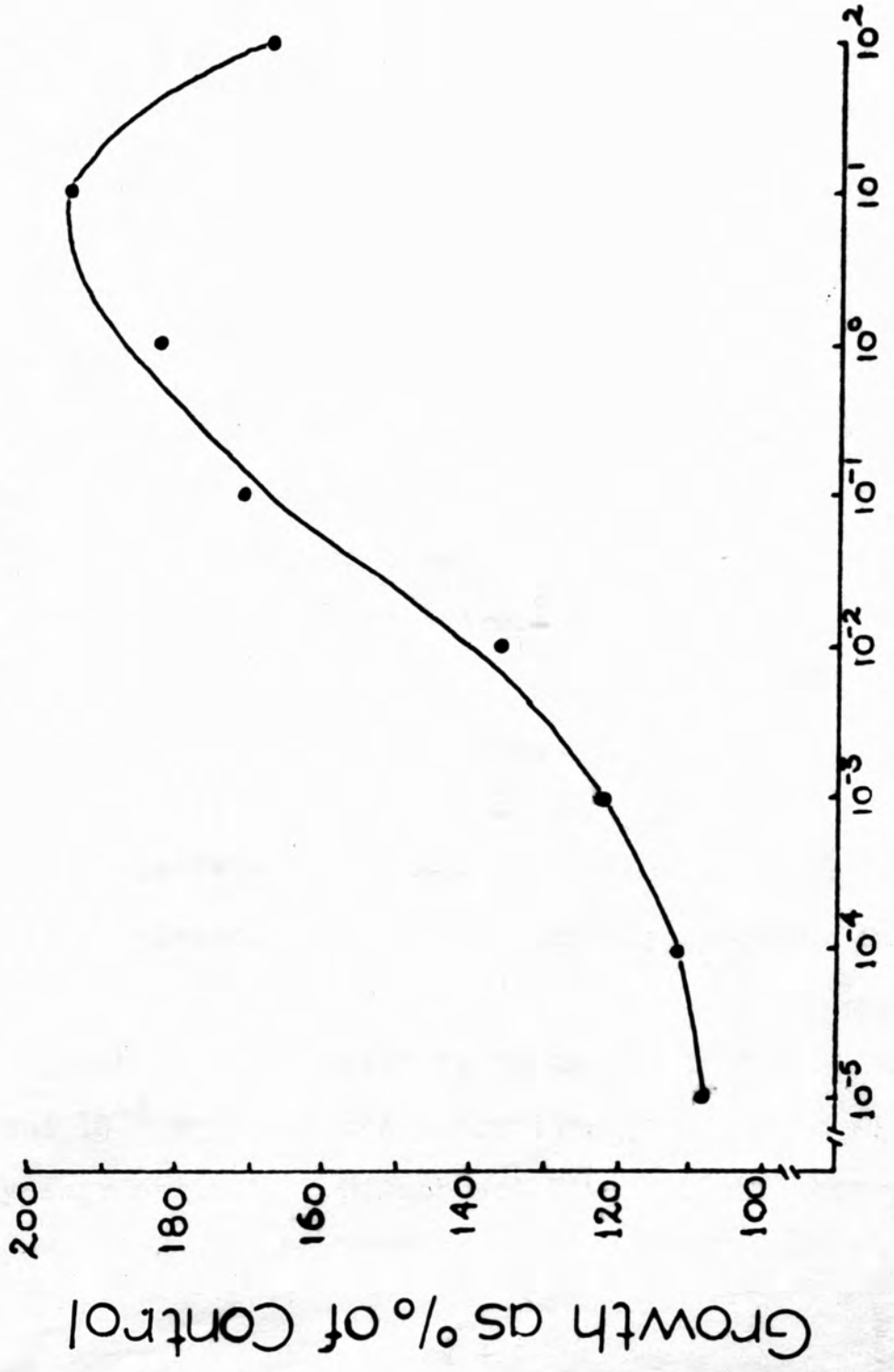
It was observed (Table I) that coleoptiles show slight inhibition due to presence of paper, but mesocotyl growth is unaffected. However, in later experiments solvent-washed papers were used whenever oat coleoptile assay was applied.

(c) Response of oat mesocotyl sections to IAA (calibration curve)

It is now a conventional method to express growth activities of unknown substances obtained from plant extracts in terms of IAA equivalents. In order to do this a standard

Fig.7. Calibration curve. Growth (as % of controls) of oat mesocotyl sections in different concentrations of IAA.

FIG. 7.


Growth as % of Control
200
180
160
140
120
100
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
IAA. mg/l.

concentration-response curve for IAA has to be prepared.

IAA solutions were prepared by dissolving known amounts of IAA in a small volume of 0.1N NaOH, neutralised with HCl and made up to the required volume in buffered sucrose solution (pH 5.0). A series of dilutions were prepared from this solution a few hours before commencement of assay. Mesocotyl sections were cut and floated in these solutions following the standard procedure described before. Chromatographic paper strips (cut to fine pieces) were included in each vial. Calibration curves prepared on different occasions have been brought together in Table 1 (appendix) and the mean curve has been presented in Fig.7. Concentration determination of a significant reading obtained from assay of chromatograms containing plant extracts has been performed by taking antilogarithms of direct readings from this curve. Results were expressed in IAA equivalents.

The high sensitivity of this assay method has been generally accepted (Nitsch and Nitsch, 1956; Bentley, 1958). It has an added advantage of not showing any response to amino acids.

The threshhold of sensitivity in this method was found to be around 10^{-4} mg/l and concentrations above 10^{1} mg/l are definitely supraoptimal. Nitsch and Nitsch (1956) however

Fig.8. Calibration curve. Growth (as % of controls) of pea root sections in different concentrations of IAA.

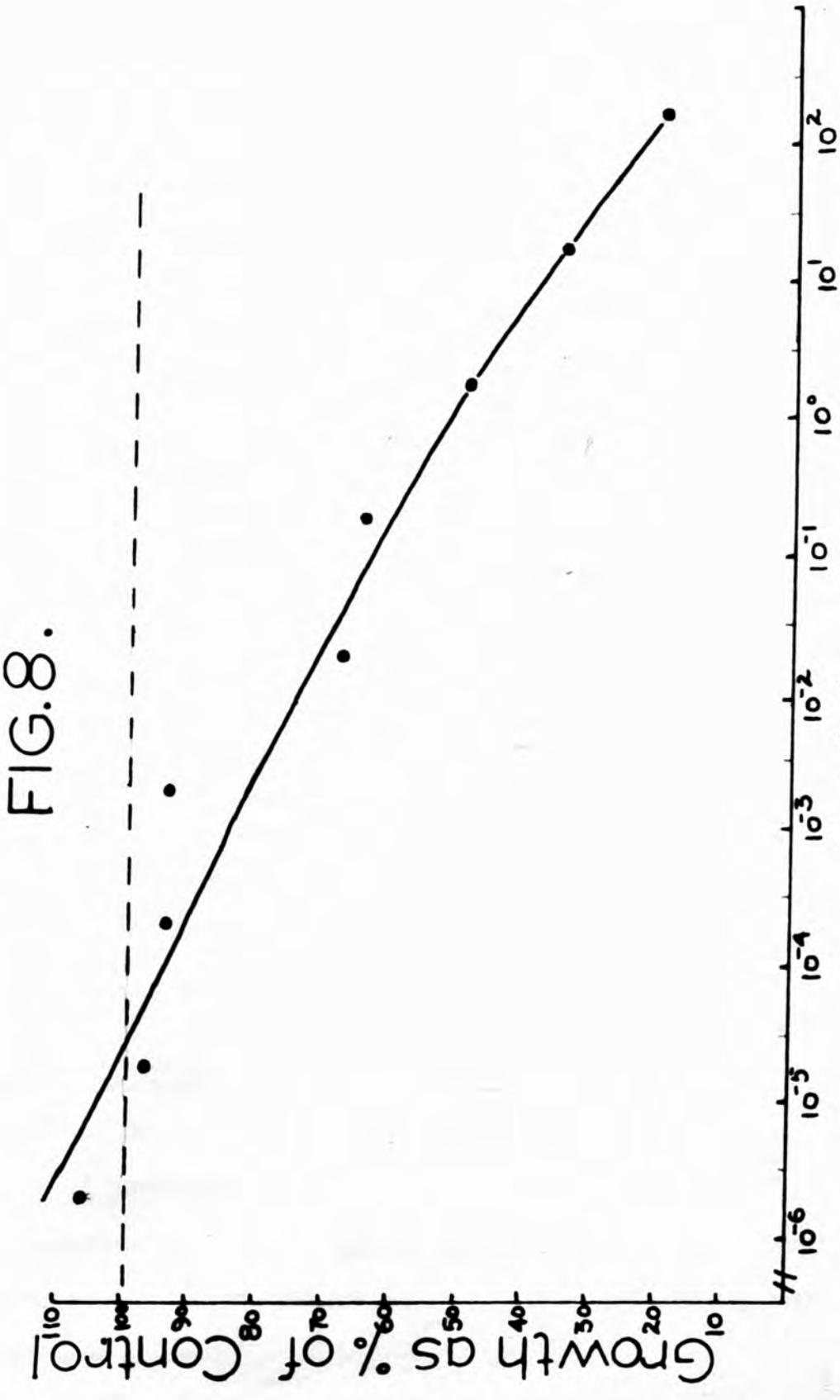

Growth as % of Control
0
10
20
30
40
50
60
70
80
90
100
IAA. mg/l.
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}

FIG. 8.

claimed that quantities up to 5×10^{-4} mgms. of IAA could be detected by this assay method.

(d) Response of pea root sections to IAA (calibration curve)

Pea root sections were excised as described before and distributed in vials containing different concentrations of IAA in 0.5% sucrose solution. The standard growth conditions of pea root sections, as stated before, was maintained.

Calibration curves prepared in course of the work have been presented in Table 2 (Appendix), and the mean curve is shown in Fig. 8.

This assay method provides a wide working range for determination of auxins. The concentration-response curve followed almost a straight line and a slight promotion in lower concentration (10^{-11}M.) could also be observed.

The wide working range for activity determination is a great advantage of this method. The main idea of applying this test was to determine the nature of growth substances in roots by an assay using the same nature of tissue.

(e) Assay and chromatography of auxin

The most important part of auxin chromatography is the estimation of unknown substances separated on paper. The possible sources of error can be due to:

(i) decomposition of growth-active compounds during the process of chromatography.

(ii) incomplete elution from paper.

(iii) biological sources of error. (i.e. Errors due to variable sensitivity of assay material towards growth substances. This problem, as mentioned before, can only be resolved by repeated experiments.)

(iv) occurrence of more than one substance at the same position on paper (can be overcome by using different solvent systems).

(v) variation in extraction efficiency. This has an important bearing on the quality and quantity of growth substances recorded in a particular investigation. Information obtained from inefficient extraction methods can be very misleading. Considerable attention has been paid to this problem in the preliminary experiments with plant extracts.

A serious obstacle in relation to these problems is that the exact chemical nature of all endogenous growth factors is not known. However, the first two sources of error have been investigated in the following experiments in relation to IAA.

1. Bioassay method

A known amount of IAA, dissolved in alcohol, was spotted on paper with a micropipette and the chromatogram was run in solvent with a suitable marker chromatogram (i.e. a chromatogram

with known amount of IAA, developed concurrently for spraying). The position of IAA on the paper was determined by the position of the visible spot on the marker (after spraying with Ehrlich's reagent). The paper was previously divided into 20 equal segments according to Rf. values and only the IAA portion of the chromatogram was assayed with the oat mesocotyl section test.

In the first few experiments considerable variations were observed in the total amounts assayed. The recovery was in the order of 65%, 45%, 85% and 88% with 1.0 µgm. of IAA. In later experiments a standard period of four hours was allowed for elution in the assay medium; and thus, the variation was minimised.

TABLE 2.

IAA known amount µgm.	IAA recovered µgm.	% Recovery	Mean (% recovery)
1.0	0.89	89.0	91.0
	0.93	93.0	
	0.99	99.0	
	0.85	85.0	
5.0	4.5	90.0	97.0
	5.2	104.0	
	5.4	108.0	
	4.3	86.0	

(table contd.)

IAA known amount µgm.	IAA recovered µgm.	% Recovery	Mean (% recovery)
0.1	0.1	100.0	105.0
	0.11	110.00	
	0.09	90.0	
	0.12	120.0	
.01	0.0113	113.0	96.0
	0.0082	82.0	
	0.0072	79.0	
	0.0110	110.0	

From the results of the experiments (Table 2) it was concluded that loss of IAA during chromatography was not a probable cause for the slight differences in recovery as recorded in different experiments. Biological sources of error were clearly indicated in experiments where supra-theoretical amounts were recorded.

This point was further investigated in the next series of experiments where bioassay was replaced by colorimetric estimation.

2. Spectrophotometric method

Known amounts of IAA made up with ethanol were spotted on paper as in previous experiments. The chromatograms were run in solvent, dried in still air and the IAA zone, as indicated by the marker spot, was cut off and eluted in 10 ml. of alcohol. After three hours of elution the alcohol was

evaporated off under reduced pressure and the residue was taken up in 2.0 ml. glass-distilled water.

In another set, solution was spotted on paper (2 x 1 cm.), dried and a similar procedure of elution was carried out omitting the chromatographic step.

Controls consisted of the same amounts of alcoholic IAA solution, pipetted directly into the bioassay tubes, dried and left to dissolve in 2.0 ml. of water for 3 hours. In all cases 1.0 ml. aliquot was taken for assay.

The IAA content was measured using Ferric-chloride / Perchloric acid reagent, the maximum absorbency being determined at 530 mμ. (see Table 3, Appendix) after 30 mins. colour development at laboratory temperature and in diffused light.

TABLE 3.

IAA cont. in 1 ml. aliquot (μgm)	Absorbencies in log. scale				
	Direct assay	Assay of eluate (cont.)	Eluate from 2x1 cm. paper	Eluate from chromatograms (replicates)	
12.5	0.21	0.21	0.20	0.21	0.21
25	0.4	0.38	0.35	0.38	0.4
62.5	0.62	0.62	0.58	0.62	0.62
12.5	0.77	0.77	0.76	0.77	0.77

From the results (Table 3), it was confirmed that no loss of IAA during the process of chromatography was possible. No attempt has been made in this experiment to study the recovery of IAA with reference to a calibration curve. The main objective, however, was to compare the absorbencies in different treatments which would indicate the loss of IAA, if there is any.

Actual limitation in the method of estimation of growth substances from paper was thought to be due to variability of materials used in bioassay.

(f) Acetonitrile soluble growth substances in pea and bean roots

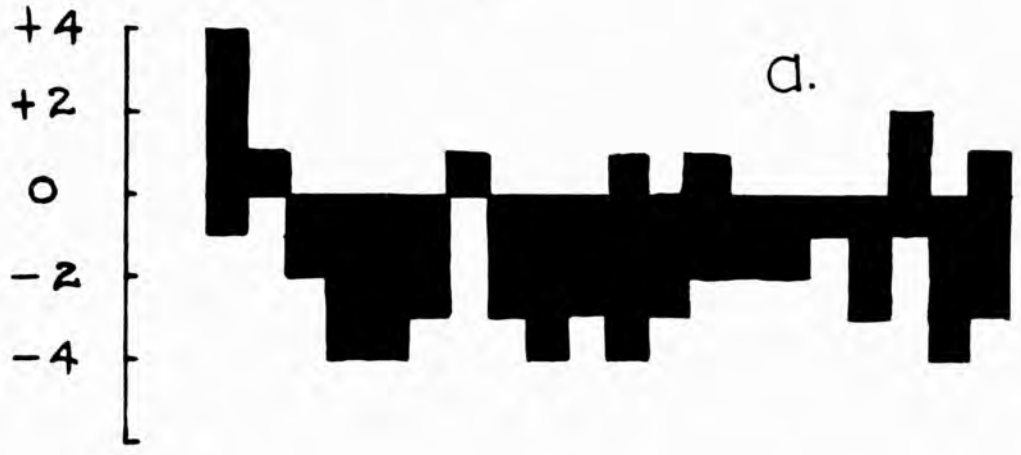
It has been claimed by Nitsch that this method gives a quantitative picture of endogenous auxins present in plant tissues.

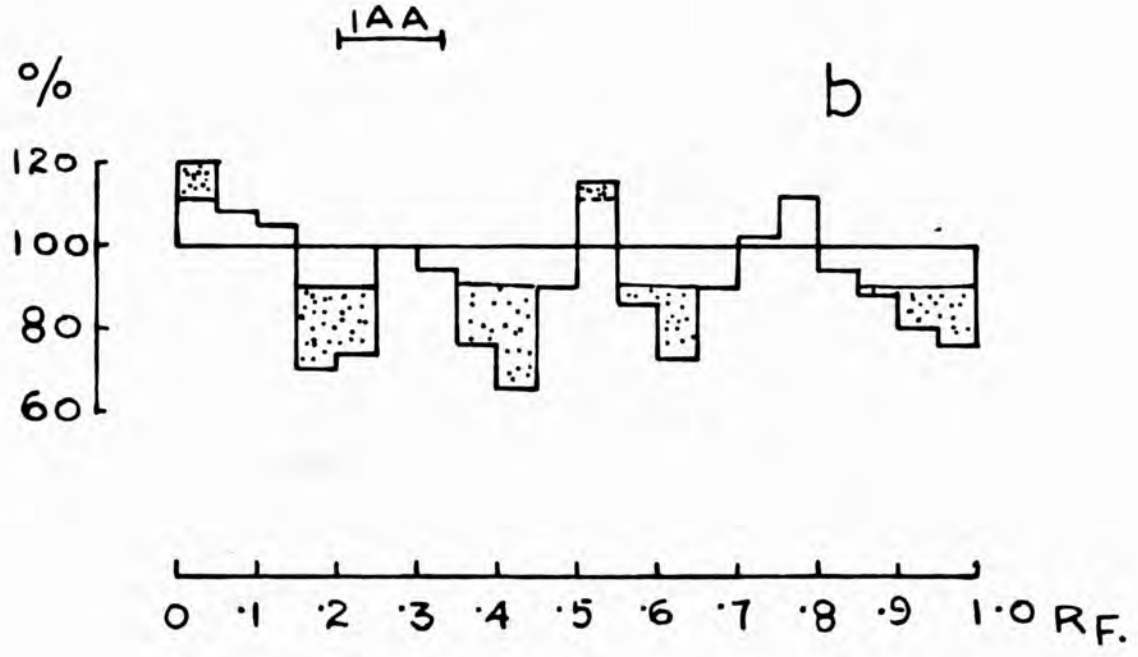
A number of experiments with ethanol extracts of pea roots were carried out following this method. Pea root section test was applied in all cases to assay the chromatograms. A sample histogram, illustrating the kind of result obtained, has been presented in Fig.9.b. This and other similar chromatograms (results of which are included in Table 4, Appendix) are summarised diagrammatically in Fig. 9.a. Here, on each occasion when a significant response was

Fig.9. a. Diagram showing integrated results of all chromatographic analyses (following pea root section assay) on acetonitrile soluble growth substances of ethanol extracts of pea roots. (Number of significant responses plotted against Rf. values.)

b. A sample histogram illustrating the pea root assay results of the chromatogram of acetonitrile soluble growth substances in the ethanol extract of 30.0 gm. of roots obtained from 3 days' old pea plants. (Growth responses plotted against Rf. values.)

FIG 9





obtained, it has been scored in its appropriate Rf. position either above (promotion) or below (inhibition) a line presenting control level of section elongation. Each square therefore presents one significant response.

The histograms revealed the following growth-active zones, with slight occasional variability:- (i) promotion, 0 - .1 Rf., (ii) Inhibition, 0.1 - 0.3 (IAA position), (iii) Inhibition, 0.35 - 0.5 Rf., (iv) Inhibition, 0.6 - 0.7 Rf., and (v) Inhibition, 0.8 - 1.0 Rf.

It was thought that there must be certain extent of mutual interference due to close position of active zones. Occasional shifting of Rf. values has produced a continuous zone of inhibition from Rf. 0.35 - 0.75 in Fig. 9.a., which in fact resulted from merger of two inhibitor as close Rf. values (as indicated from Fig. 9.b.). There are also indications of occurrence of a promotor at Rf. 0.85 - 0.9 (Fig. 9.a.), which produced variable responses.

The stimulation of root sections below the IAA position was possibly due to "accelerator α" of Bennet-Clark and Kefford (1953), recently suggested to be Indoleaspartile acetic acid (Bennet-Clark et al. 1959). The inhibition at the IAA region was consistent in all chromatograms. Growth

inhibition due to the presence of another substance immediately after IAA was also observed in all chromatograms. But the next active zone, which occurred around 0.6 - 0.7 Rf. value, was variable in its activity. It might be due to partial solubility of the substance in acetonitrile or could be due to interference of other substance occurring at same Rf. value. Another inhibitor at higher Rf. value (around 0.8 - 1.0) was observed in all cases excepting in one chromatogram.

In the acid fraction of pea root extracts Audus and Thresh (1956) detected IAA, inhibitor β (Rf. 0.7) coming just after IAA and another inhibitor occurring near the solvent front (Rf. 0.9). All these three substances were detected in the present investigation with slight changes in Rf. values. In addition to these, one promotor occurring before IAA and one inhibitor occurring between inhibitor β and the inhibitor near solvent front were also observed.

The results of Audus and Gunning (1958) are strictly comparable with results presented here, because experimental conditions were identical in both cases. They used Avena coleoptile and mesocotyl and wheat coleoptile test to investigate acetonitrile soluble growth substances in pea roots. Their AP(i) (Rf. 0.- 0.15), AP(ii) (Rf. 0.15 - 0.3)

and AI(i) (Rf. varying from 0.3 to 0.5) are directly comparable with substances detected here by pea root assay. They also observed slight promotion in Avena tests in regions between Rf. 0.5 - 0.6 (APiii). In pea root assay inhibition was recorded at the same Rf. value but its expression was rather variable. Similar inconsistency was noted by previous authors when they applied the wheat coleoptile test. Above 0.7 Rf. value Audus and Gunning obtained interference, possibly due to occurrence of promoting and inhibiting substances at close Rf. values. Still in Avena coleoptile assay, they could demonstrate an inhibitor above the region of Rf. 0.7 (AI(ii). In pea root assay a consistent inhibition was recorded at 0.8 - 1.0 Rf. value with indications of the occurrence of a promotor at Rf. 0.85 - 0.9.

In the next series of experiments acetonitrile soluble growth substances in broad bean roots were investigated. From oat mesocotyl assay of duplicate chromatograms (Figs. 10.a. and b.) the following growth active zones were recorded: (i) Promotion, Rf. 0 - 0.25; (ii) Promotion, Rf. 0.4 - 0.6; (iii) Promotion, Rf. 0.65 - 0.8; (iv) Promotion, Rf. 0.85 - 1.0.

Almost a similar picture of growth substances was observed when oat coleoptile assay was used (Figs. 11.a. and b.).

Fig.10. Chromatograms of acetonitrile soluble growth substances in ethanol extracts of broad bean roots. (Growth responses plotted against Rf. values.)

a. Oat mesocotyl section assay of the chromatogram of 51.0 gm. of root tissue obtained from 14 days' old plants.

b. 58.0 gm. of root tissue was used in the experiment. Other treatments same as in (a).

FIG.10.

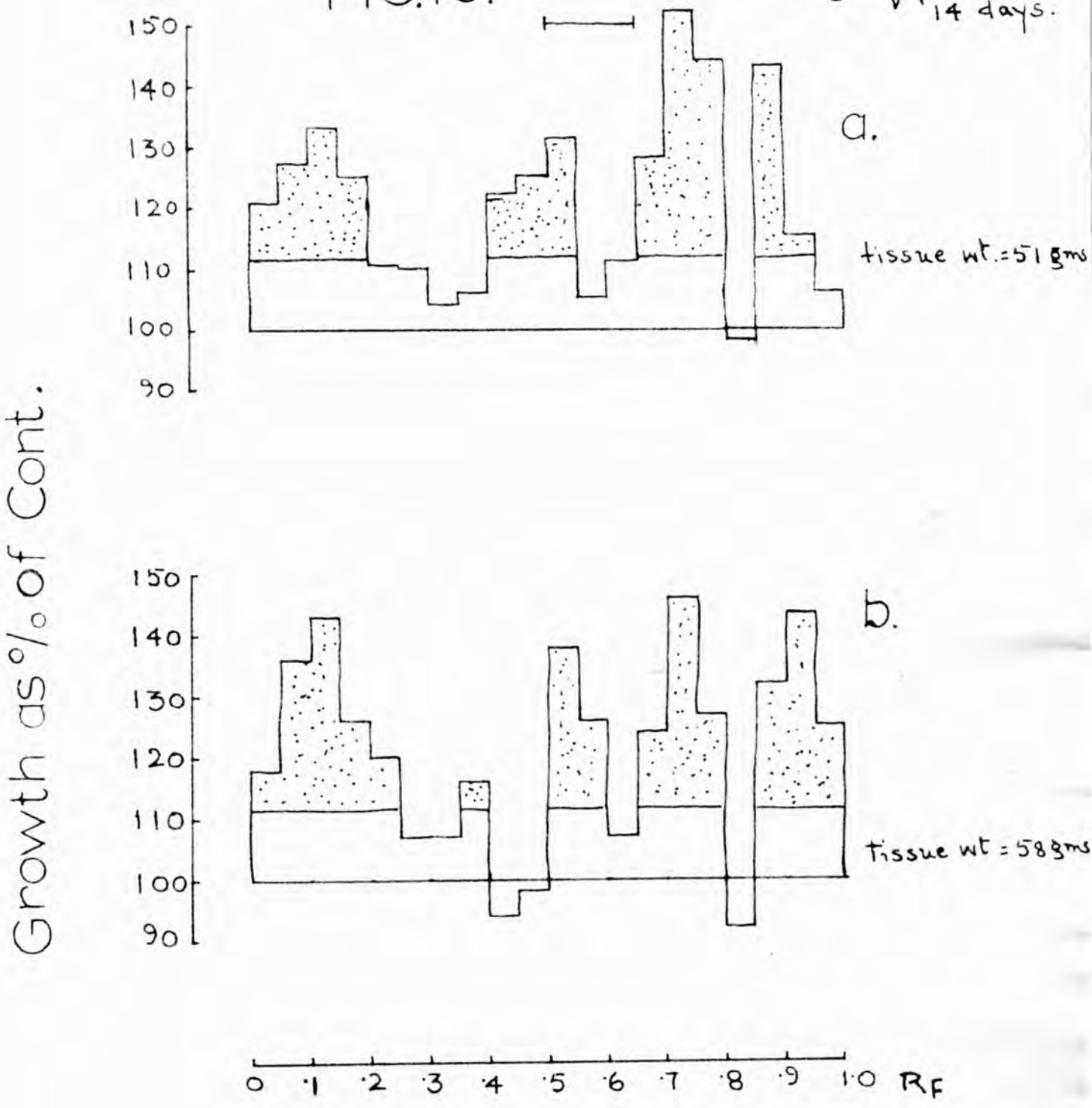
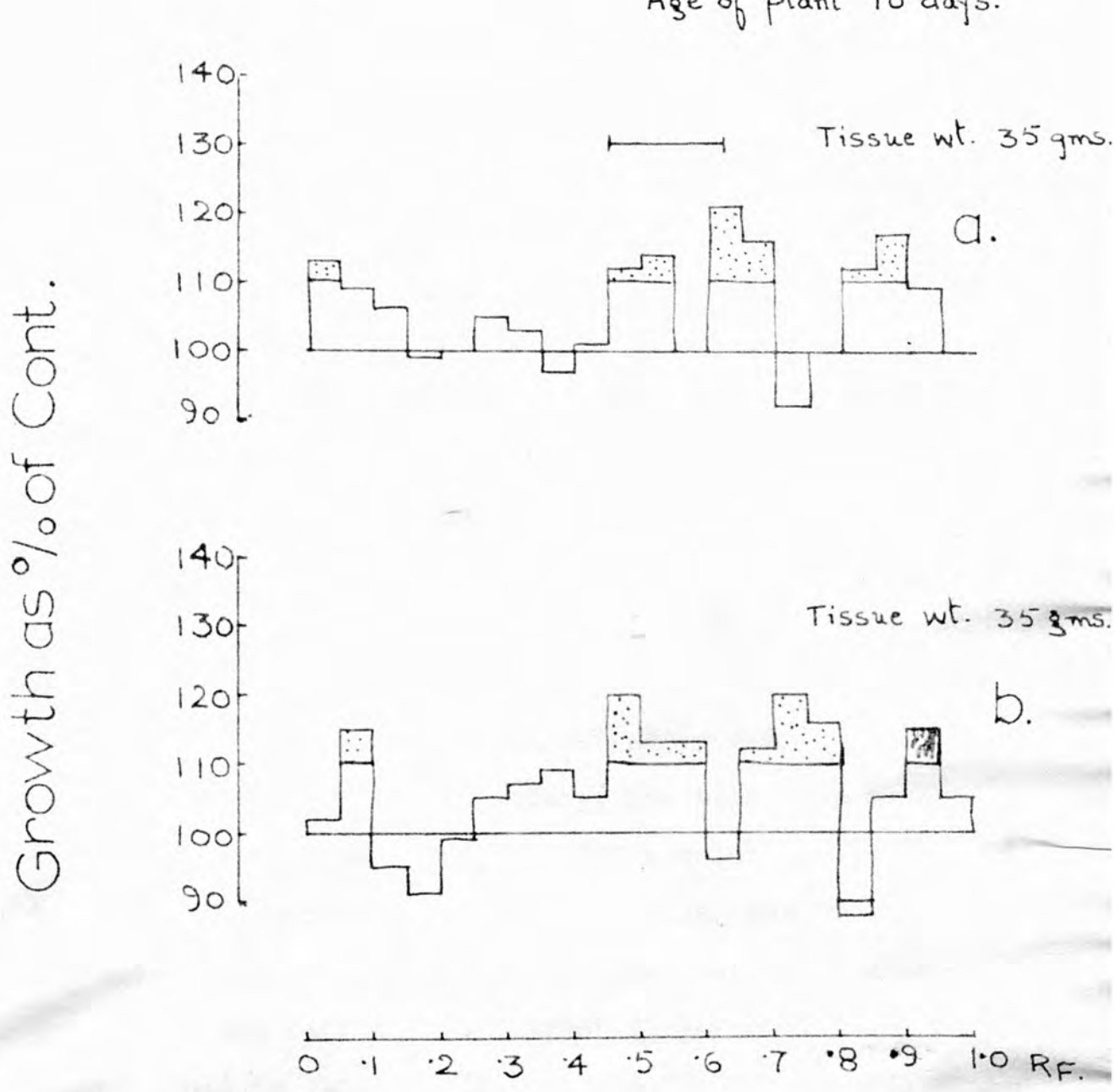

Age of plant-
14 days.
a.
tissue wt.=51 gms
b.
tissue wt = 58 gms
Growth as % of Cont.
150
140
130
120
110
100
90
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF

Fig.11. Acetonitrile soluble growth substances in ethanol extracts of broad bean roots. (Growth responses plotted against Rf. values.)

a. Oat coleoptile section assay of the chromatogram of 35.0 gm. of root tissue obtained from 10 days' old plants.

b. Same treatment and tissue weight as in (a).

FIG. 11.


Age of plant 10 days.
Growth as % of Cont.
140
130
120
110
100
90
Tissue wt. 35 gms.
a.
Tissue wt. 35 gms.
b.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

It may be observed that Rf. value of IAA has shifted significantly, from that noted before. However, this value of IAA remained fairly constant in the rest of the investigation. This variation in Rf. value of IAA has been mentioned before with reference to chromatographic method. (Ch. III.C.)

The investigations of Kefford (1955) on acid fraction of similar material have shown three growth substances: accelerator α, IAA and inhibitor β. But, in the present investigation the first two of these were detected, along with two other promotors. In oat coleoptile assay slight (although significant) inhibition at 0.8 Rf. value was observed in one of the chromatograms. (Fig. 11.b.)

From a parallel investigation carried out in this laboratory (Audus and Gunning, 1958), it was noted that acetonitrile method was not quantitative for IAA. On the basis of their findings no attempt has been made to express the growth activities of acetonitrile soluble substances in terms of IAA equivalents. Nevertheless, the results presented here are of qualitative interest and a direct pointer to the fact that different pictures of endogenous auxins could be obtained following different methods of extraction and purification.

Therefore, the investigation was logically directed towards development of a better method of purification.

(g) Investigations on chromatographic purification

(i) Development of the method

Chromatography of crude extracts in water and subsequent elution of paper, excluding the initial spot, has been thought to be an efficient method of purification (Nitsch, 1955). This method was investigated in detail in the following experiments.

1. The paper was run in distilled water with known amounts of IAA and IAN, dried and sprayed with $FeCl_3$/Perchloric acid reagent. The Rf. values of these substances are shown in Table 4.

Table 4.

Substance	Separate Rf.	In mixture, Rf.
IAA	.85 - .92	.82 - .92
IAN	.31 - .5	.32 - .49

2. Ether as a solvent for elution.

Papers with known amounts of IAA were run in distilled water and dried. The IAA zone was cut off from the paper and eluted with three changes of 10 ml. of ether. A time period of 15 mins. was allowed for each elution. The ether washings were combined, evaporated to dryness and taken up in water. The recovery was studied by spectrophotometric

method after colour production with $FeCl_3$/Perchloric acid reagent. The following results (Table 5) were obtained:-

Table 5.

Known amount of IAA mgm.	Absorbency in replicate controls		Absorbency in eluate obtained	Absorbency in eluate obtained by 18 hr. soaking in ether at -15°C.
8.32	0.195	0.168	-	-
16.65	0.38	0.35	0.04	-
25	0.49	0.45	0.03	-
50	0.72	0.69	0.1	0.055

A comparison of figures in columns 2, 3 and 4 directly indicate that the recovery of IAA from paper was very poor.

In all cases the paper had large amounts of IAA absorbed to it. When the papers (after ether elution) were treated with $FeCl_3$/Perchloric acid reagent, visible colour due to IAA could be observed.

3. Results with other solvents.

In the next experiment, elution of IAA and IAN from water chromatograms was tried with ethanol, methanol, distilled water and acetonitrile.

IAA and IAN were spotted on paper (25 µgms. of each), run in distilled water, and the active zones, as indicated by

a marker chromatogram, were cut off. The paper bits were left with different solvents in separate boiling tubes overnight at 2°C. in the dark. Later, papers were taken out, dried and sprayed with suitable reagents for colour production.

The score has been shown in Table 6.

Table 6.

Substance	Original	Ethanol	Methanol	H_2O	CH_3CN
IAA	****	-	-	-	**
IAN	****	-	-	-	-

It showed that all these solvents excepting acetonitrile could remove IAA and IAN from water chromatogram.

For practical purposes, however, ethanol was thought to be useful and its merits were further investigated in the next experiment.

4. IAA (25 µgms.) was spotted on paper and run in distilled water as in previous experiments. The IAA zone was cut off from the rest of the paper and eluted with 20 mls. of ethanol. Elution was carried out for 1, 2 or 3½ hours on an electric shaker in the dark at room temperature. The paper was taken out of the tube, washed and the alcohol was evaporated off to dryness under reduced pressure. It was

taken up again in 1 ml. of distilled water and the tube was left on the shaker for one hour. Recovery of IAA was tested spectrophotometrically at room temperature, allowing 30 mins. for colour development with $FeCl_3$/Perchloric acid reagent. Results are shown in Table 7.

Table 7.

No. of observations	Absorbency. Cont. IAA soln. 25 µgm.	Absorbency. 1 hour shaking	2 hour shaking	3½ hour shaking
1.	0.40	0.249	0.259	0.40
2.	0.40	0.249	0.259	0.40
3.	0.40	0.250	0.360	0.39
4.	0.40	0.250	0.360	0.40

From these observations it was concluded that ethanol could be safely used for all elution purposes from chromatograms

(ii) Water chromatography of plant extracts

Attempts were made at the next stage to purify plant extracts following this method.

In order to test the efficiency of this method and to compare it with acetonitrile purification method, triplicate pea root extracts were prepared from 7 days' old plants, each containing 42.0gms. of tissue. One of these extracts was purified by acetonitrile method and the other two were

Fig.12. Assay results of chromatograms obtained following water-chromatographic and acetonitrile purification methods of ethanol extracts of pea roots. (Growth responses plotted against Rf. values.)

a. Pea root section assay of the chromatogram obtained following water-chromatographic purification method. 42.0 gm. of pea roots from 7 days' old plants were used for extraction. 1.0 µgm. of IAA was added at the time of maceration of frozen tissue.

b. Same treatment as in (a), but without IAA. Only the IAA zone of the chromatogram assayed with pea root section test.

c. Pea root section assay of the chromatogram obtained following acetonitrile purification method. Similar tissue as in (a) and (b).

FIG.12.

Growth as % of Cont.
IAA
a
b
c
120
90
80
60
40
100
80
60
40
120
100
80
60
0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0 RF.

subjected to water chromatographic purification (method previously described). In one of the latter extracts (used in water chromatography), 1.0 µgm. of IAA was added, at the time of maceration of frozen roots.

In this experiment at least, the movement of water (on water chromatogram) was fairly rapid (about 18 cms. from starting line in 18 hours). The pea root section test was applied to assay the final chromatograms obtained in this experiment. The results are presented as histograms in Fig. 12 (a., b. and c.). Only the IAA zone of the control chromatogram (Fig. 12.b. - water chromatographic purification without IAA) was assayed.

The results showed that purification following water chromatographic method allowed three inhibitors (Rfs. around 0.15, 0.45 (IAA zone) and 0.75) to show up in final assay. But, in acetonitrile method, four inhibitors could be detected, which further confirmed the results obtained in previous experiments (Fig. 9).

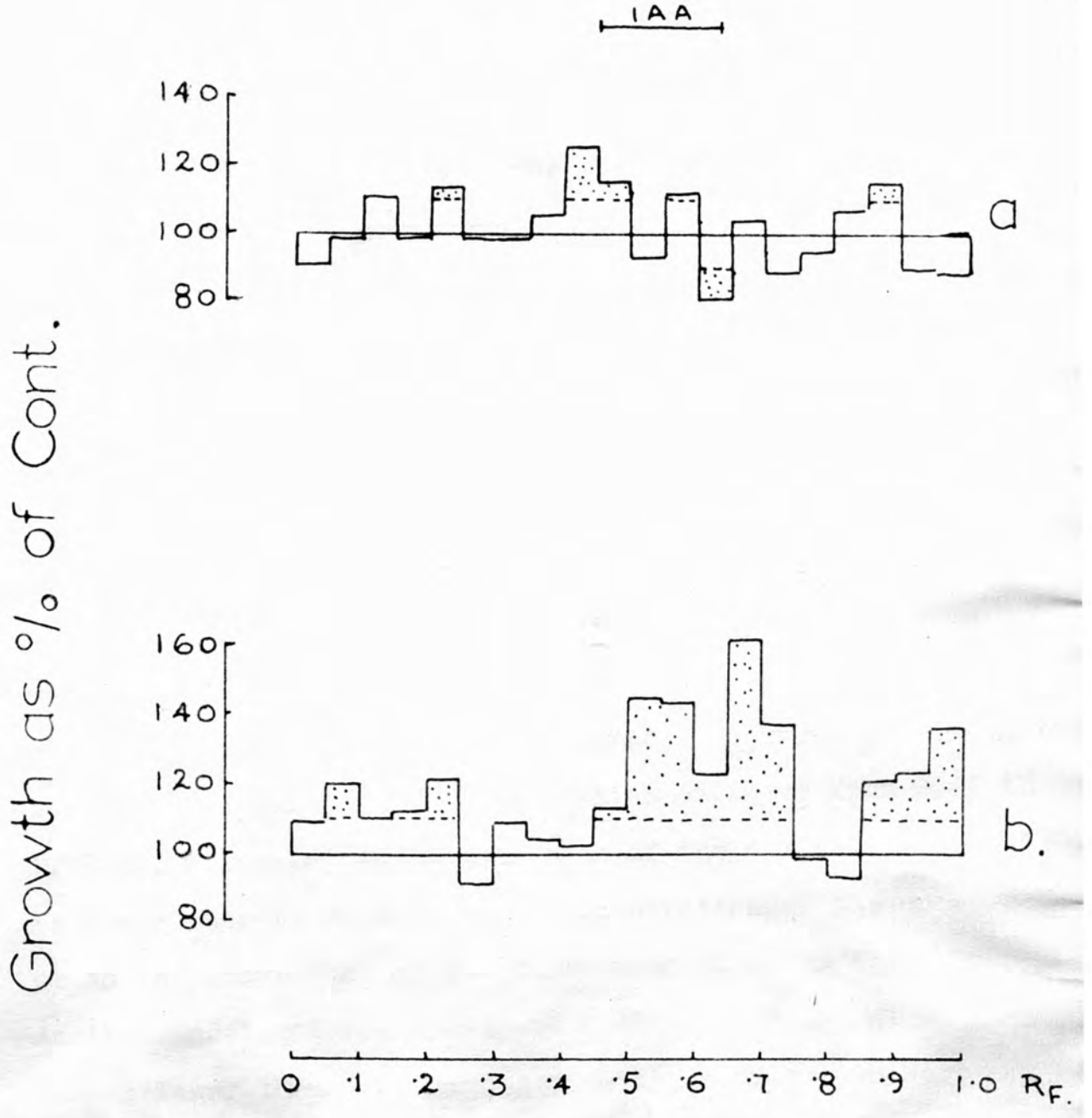
From quantitative estimation (by taking antilogarithms of direct readings from calibration curve - Fig.8) of the substance occurring at the IAA region of water chromatograms, 0.525 µgm. was noted in the control and 1.69 µgm. in the IAA enriched extract (1 µgm. of IAA was added previously). This

Fig.13. Growth substances in root tips and root stumps of peas, obtained following water chromatographic purification method.

a. Oat mesocotyl section assay of the chromatogram of 4.0 gm. of root tips (regions 1.0 cm. from the apex) obtained from 4 days' old pea plants.

b. Oat mesocotyl section assay of the chromatogram of 61.0 gm. of root stumps obtained from 4 days' old plants.

FIG.13.


IAA
140
120
100
80
a
160
140
120
100
80
b.
Growth as % of Cont.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

supra-theoretical field could be attributed to the biological sources of variation. In the acetonitrile purification method, the quantity of the substance at the IAA zone was 0.26 µgm. - less than that observed in control water chromatogram.

In the next experiment, the nature of the substances obtained by water chromatographic method was tested with oat mesocotyl section assay. Two separate extracts of 61.0gms. of root stumps and 4.0gms. of root tips were prepared from 4 days' old dark-grown peas. The resulting histograms are shown in Fig. 13.a.(tip) and b.(stump). In the analysis of the tip significant stimulation was obtained at the IAA region. Besides that, slight stimulations were observed near 0.2, 0.55 and 0.85 Rf. values and slight inhibition near 0.6 Rf. In the analysis of the stumps, however, the zones of stimulation were more marked. A large promotion was observed near the IAA zone, which extended from 0.45 to 0.75 Rf. values. The double-peak of the active zone could be due either to supraoptimal concentration of the substance or to the occurrence of two substances at close Rfs. Another accelerator occurred at 0.85 - 1.0 Rf. At Rf. values lower than IAA, slight promotions of growth were obtained around 0.05 and 0.2 Rf. values.

Quantitative estimation of growth substances was not attempted in consideration of certain drawbacks of this method. It was soon found out by repetition of experiments that this procedure was not suitable as a standard method of investigation. The method was not pursued any further, with a view to the following disadvantages of the method:

1. The loading of water chromatogram with gummy-water-residue was very difficult. It took hours to dry the extract on paper.

2. The movement of water was extremely slow due to the presence of residues on paper. On occasions it took 2 - 3 days to travel less than 10 cms. from the starting line. The running time, however, varied according to the amount of impurity applied on paper. Although the chromatograms were run in the dark, it was not desirable to leave them exposed to air for a long time. This difficulty could, however, be averted by running in an atmosphere of Nitrogen, but uncertain experimental conditions were sure to handicap the efficiency of this method.

3. It was again observed that impurities at the starting line tend to separate out with water into two portions. These were:

(a) Impurities that remained stationary at the starting line; and

(b) Water soluble impurities that cover a large area on the paper.

This spreading of impurities was a serious disadvantage.

4. Elimination of starting line and the spreading zone of impurities might be dangerous, because substances showing low Rf. values in water could also be excluded.

2.(a) Investigations on water-soluble and ether-insoluble fraction from ethanol extract

So far, most of the chromatographic investigations in the field of auxin research were confined to acid ether soluble fraction of the extract. As a result most of the substances occurring in this fraction have been extensively studied. On the contrary, we have fragmentary information about the growth substances occurring in other fractions of the extract. Physiological interpretation of growth processes in plants can be very misleading if the observations are only confined to a single fraction.

From recent work of Bentley, Housley and Britton (1955) and Audus and Gunning (1957), there is a growing realisation that growth active compounds can occur in large quantities in the ether-insoluble aqueous fraction of the crude extract. These findings have made problems related to root growth more complex than ever.

Fig.13. (contd.)

Oat mesocotyl assay of chromatograms of water soluble, ether-insoluble fractions of ethanol extracts of broad bean roots. Aqueous residue equivalent to 7.03 gm. of fresh wt. of tissue was used for final extraction. (Extract from 11 days' old plants - Growth responses plotted against Rf. values.)

c. ethanol-soluble.

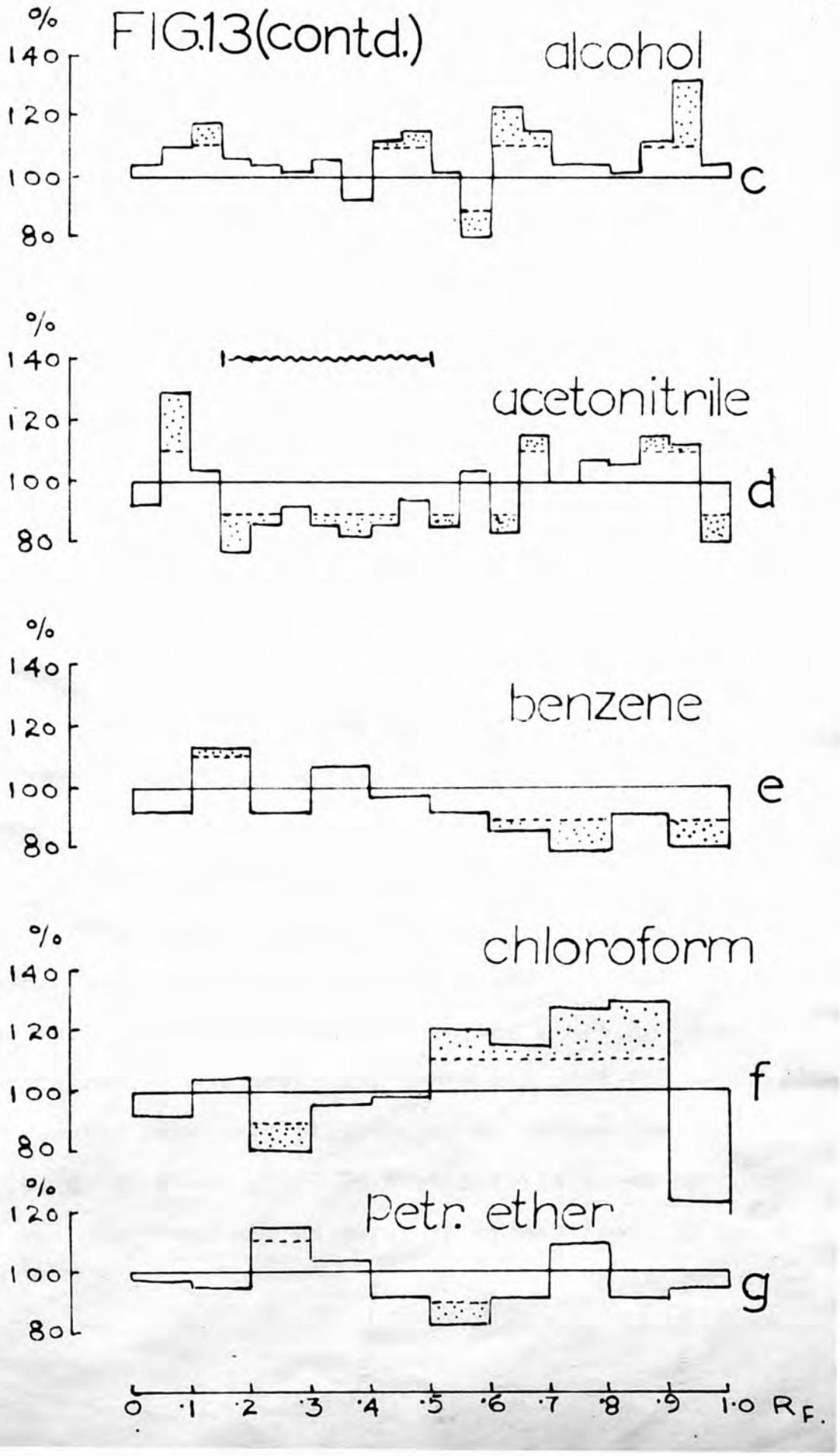
d. acetonitrile-soluble.

e. benzene-soluble.

f. chloroform-soluble.

g. petroleum ether-soluble.

FIG.13(contd.)


%
140
120
100
80
alcohol
c
%
140
120
100
80
acetonitrile
d
%
140
120
100
80
benzene
e
%
140
120
100
80
chloroform
f
%
120
100
80
petr. ether
g
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

While the work of Audus and Gunning was in progress in this laboratory, a parallel investigation was carried out by the author on broad bean roots in order to substantiate their findings.

In the preliminary experiments the main objective was to find out a suitable solvent for extraction of these growth active compounds. In the first experiment water soluble fraction was prepared (method has been described under Material and Method) of roots obtained from 11 days' old roots. The procedure has already been described. The neutral water-fraction was evaporated off to dryness under reduced pressure and the residue (1.42 gm.) was taken up in 1.5 ml. of distilled water. Aliquot parts (0.1 ml.) of this thick brown syrup were extracted separately with acetonitrile, chloroform, alcohol, benzene and petroleum ether (3.0 ml. of each) for 18 hours at 2°C. in the dark. At the end of this period the solvent was decanted off, centrifuged and the clear supernetent was brought down to a small volume under low pressure. It was then applied to the starting point of the chromatogram. Oat mesocotyl assay was used for bioassay.

The results have been illustrated as histograms in Fig. 13 (c, d, e, f and g). In acetonitrile large amount of impurity was dissolved and on paper it spread from .15 to .5 Rf

Fig.13. (contd.)

Oat mesocotyl assay of chromatograms of water soluble, ether-insoluble fractions of ethanol extracts of broad bean root. Aqueous residue equivalent to 11.3 gm. of fresh weight of root tissue was used for final extraction. (Extract from 15 days' old plants) (Growth responses plotted against Rf. values.)

h. and i. Ethanol-soluble.

j. and k. Chloroform-soluble.

FIG13(contd.)

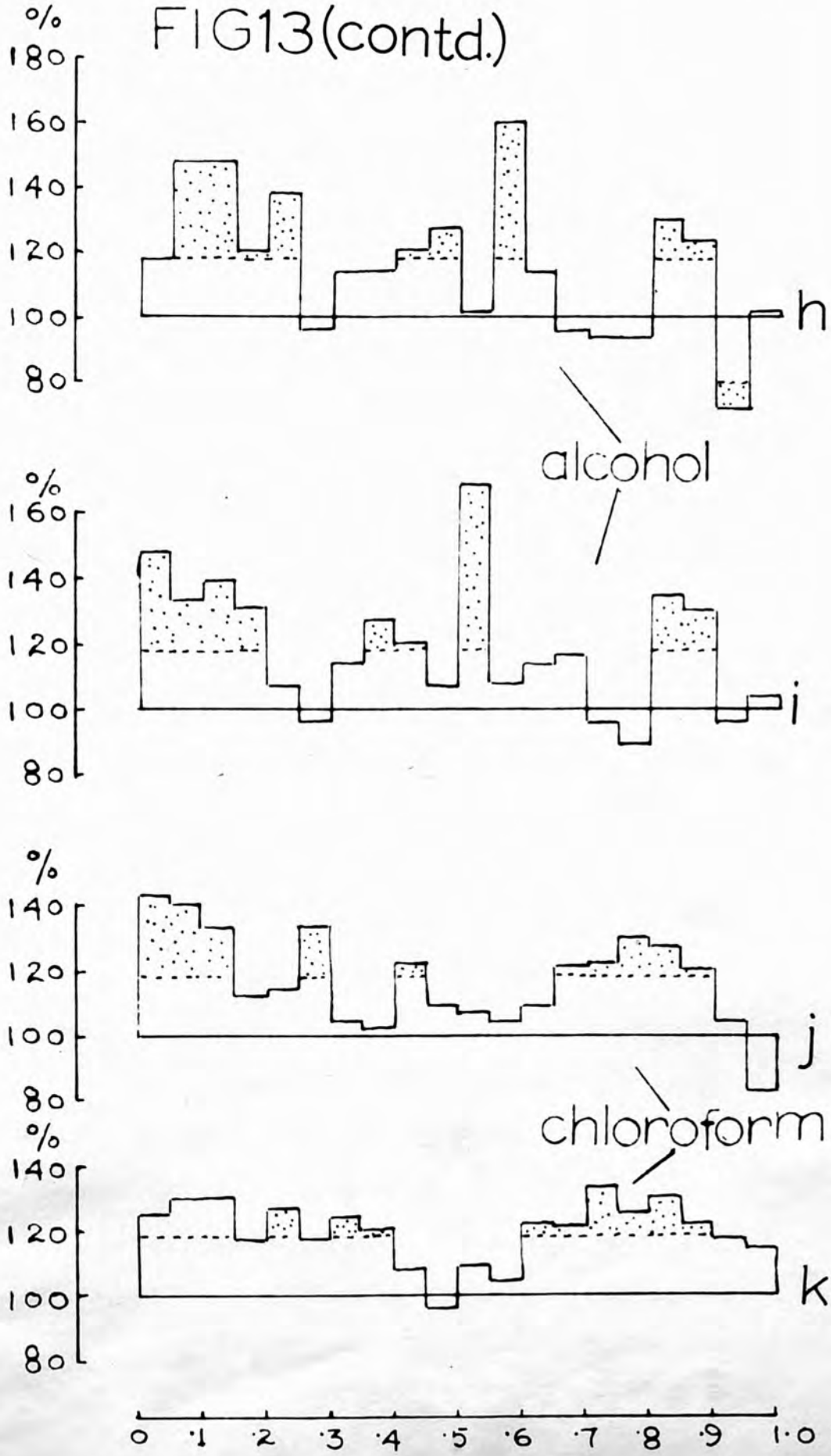

%
180
160
140
120
100
80
h
alcohol
%
160
140
120
100
80
i
%
140
120
100
80
j
chloroform
%
140
120
100
80
k
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.13. (contd.)

Activities of water-soluble growth substances in different bioassay methods. Aqueous residue equivalent to 10.0 gm. of fresh weight of root tissue was used in each experiment. (Growth responses plotted against Rf. values.)

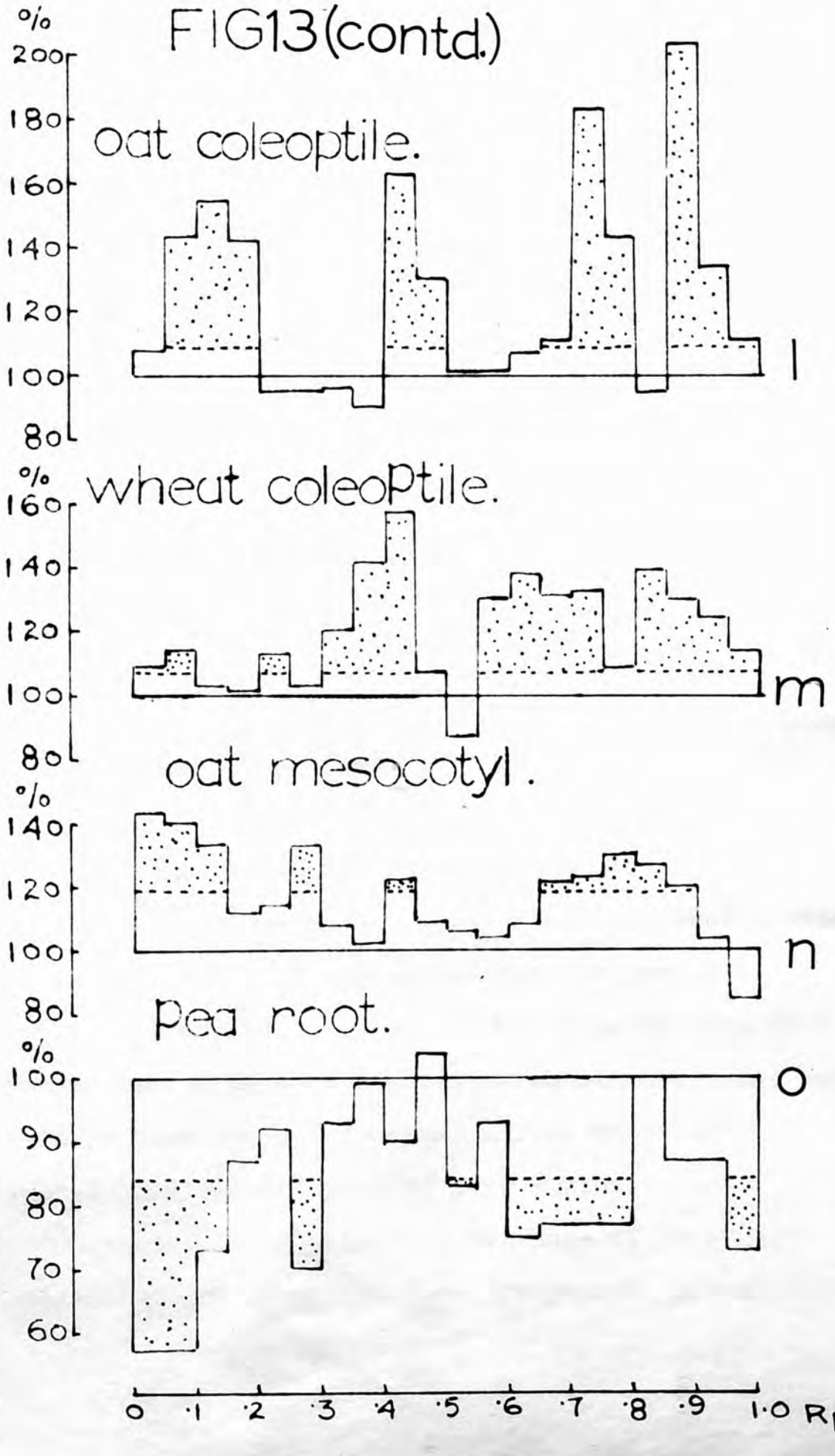
l. oat coleoptile section assay of chromatogram.

m. wheat coleoptile section assay of chromatogram.

n. oat mesocotyl section assay of chromatogram.

o. pea root section assay of chromatogram.

FIG13(contd.)


%
200
180
160
140
120
100
80
oat coleoptile.
l
%
160
140
120
100
80
wheat coleoptile.
m
%
140
120
100
80
oat mesocotyl.
n
%
100
90
80
70
60
pea root.
o
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Marked inhibition of oat mesocotyl sections was observed in this zone. The chromatogram containing chloroform extract showed unsatisfactory separation and it was repeated in the next experiment. The alcohol extract on chromatography presented four promotors and this was considered a better solvent than benzene and petrolium ether.

In the next experiment duplicate alcohol and chloroform extracts were chromatographed and the resulting histograms have been illustrated in Fig. 13 (h, i, j and k). In both the solvents four promotors were detected. In later experiments, however, alcohol purification was used as a standard method. In the foregoing experiments syrup equivalent to only 7.03 gms. of roots was used.

At this stage it was felt that the growth activities of these substances towards different assay methods should be studied. The results of oat coleoptile section, oat mesocotyl section, wheat coleoptile section and pea root section tests of water soluble growth substances are presented in Fig. 13 (l, m, n and o). It was interesting to note that these substances acted as promotors in shoot assay, but as inhibitors in root test. In other words, their biological activities fulfil the criteria of true auxins.

A similarity of chromatograms to those of Britton et al. (1956) and Audus and Gunning (1958) prompted an investigation

Fig.13. (contd.)

Chromatograms from the eluates of a primary chromatogram of the water soluble, ether insoluble fraction of an ethanol extract. (Growth substances plotted against Rf. values.) Oat mesocotyl assay was used for the assay of chromatograms.

p. Primary chromatogram (from aqueous residue equivalent to 15.6 gm. of fresh weight of root tissue).

q. Chromatogram obtained from elution and rechromatography of P_1 zone of primary chromatogram.

r. Chromatogram obtained from elution and rechromatography of P_2 zone of primary chromatogram.

s. Chromatogram obtained from elution and rechromatography of P_3 zone of primary chromatogram.

t. Chromatogram obtained from elution and rechromatography of P_4 zone of primary chromatogram.

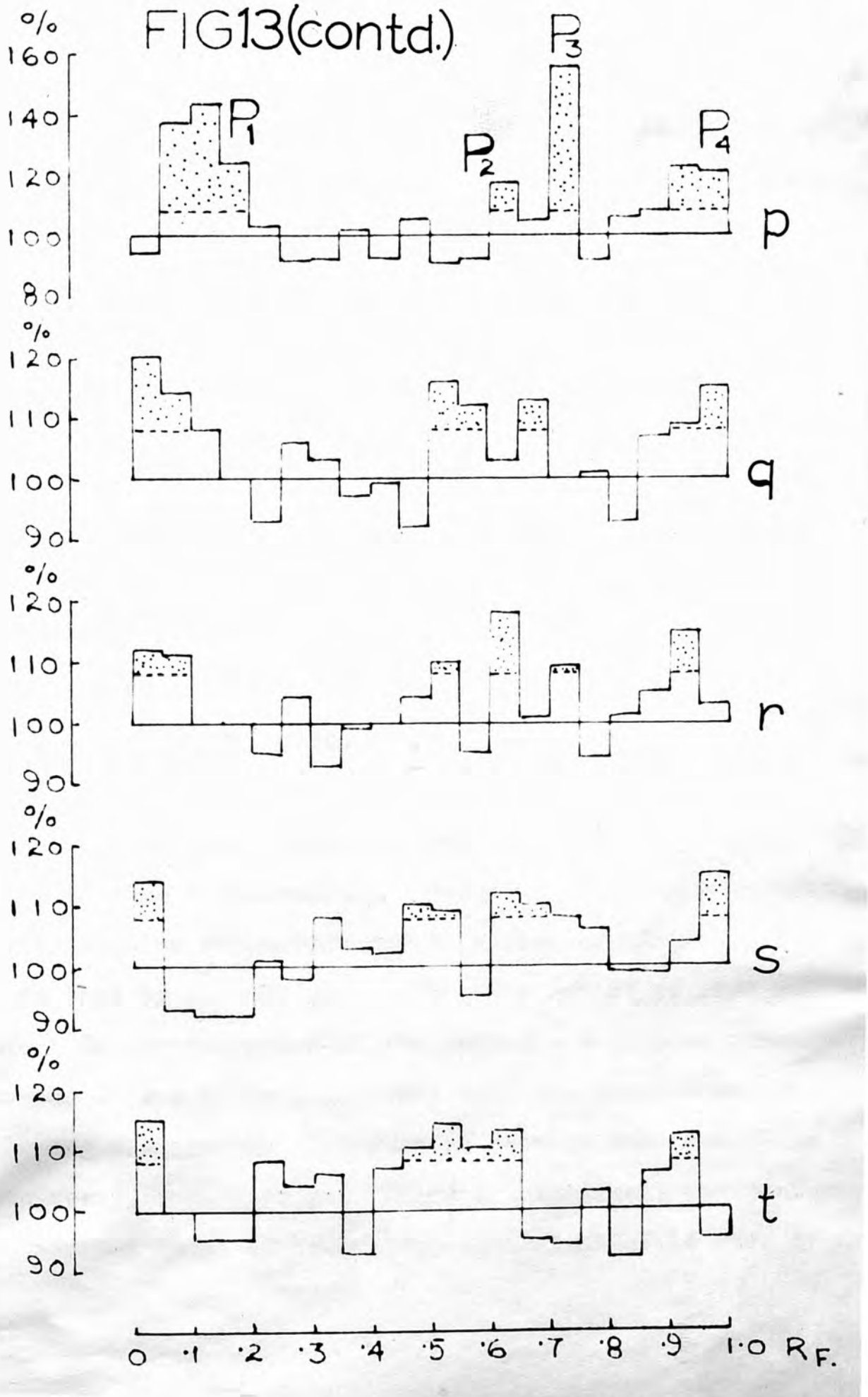


FIG13(contd.)
%
160
140
120
100
80
P1
P2
P3
P4
p
%
120
110
100
90
q
%
120
110
100
90
r
%
120
110
100
90
s
%
120
110
100
90
t
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

on stability of these substances to elution and rechromatography. Two separate spots containing 11.3 and 5.6 gms. of ethanolic extracts were applied on the same paper. After development the paper was halved and one of them (5.6 gm. extract) was assayed with oat mesocotyl test (Fig.13, b). Appropriate growth active zones from the second chromatogram were eluted in absolute ethanol following methods previous described. The eluates were concentrated and rechromatographed in the same solvent system and assayed with oat mesocotyl test. The results of elution and rechromatography of promotors, P1, P2, P3 and P4 of the marker chromatogram (Fig. 13.b) are presented in Figs. 13.q, r, s and t respectively. The results show clearly that each of these substances broke down and gave rise to activities at its own and other three positions.

From the distinct peaks of histograms of the eluates it would appear that the interconversion does not occur during the chromatographic separation. Audus and Gunning concluded from two-dimension chromatography of water soluble extract of pea roots that break down phenomena could not be related to elution. In consideration of the unstable nature of these substances, it was thought possible that the break-down could occur during the process of drying in between the runs. In order to verify this fact the following experiment was performed: Alcohol extract obtained from syrup equivalent of 14 gms. of

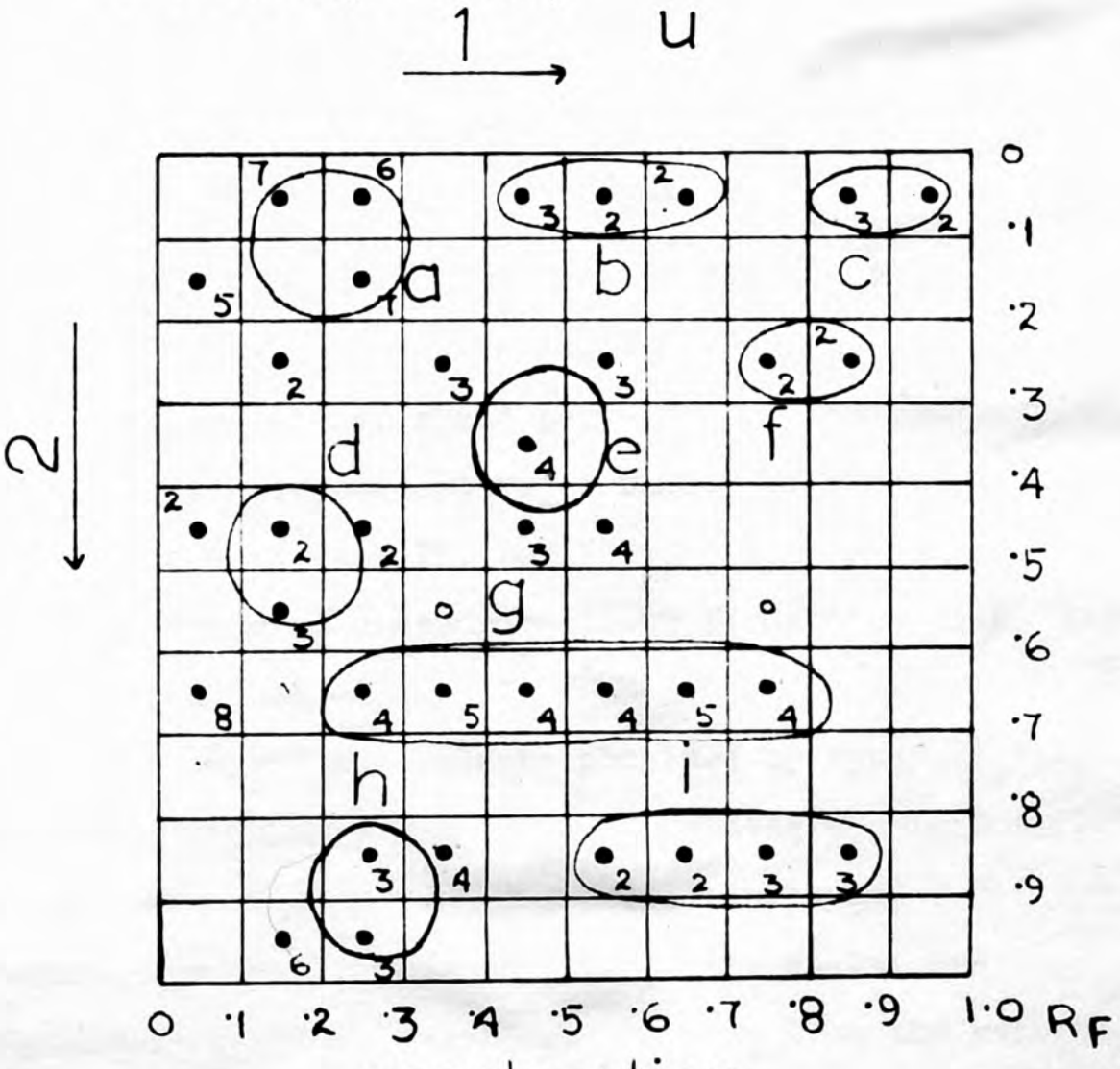
root tissue was spotted on a two-dimension chromatogram. Known amounts of IAA and IAN were spotted on a similar sheet of paper. Both the papers were run in Isobutanol : Methanol : water, in the same tank. In the first run (after 4 hrs. equilibration) solvent-front travelled about 24 cms. in 12 hours in both papers. The second run in the same solvent was started immediately afterwards without allowing the papers to dry. The second run lasted for 11 hours. After this the papers were taken out of the tank and dried in still air. The marker chromatogram with IAA and IAN was sprayed with Ferric/Perchloric reagent. Clear separation of both the substances was observed after colour development. Assuming that the IAN has travelled at .8-.95 Rf. (standard position of IAN in this solvent system) in the second run the position of the solvent front was determined. The position of the solvent front in the second run, thus determined, came out to be 19 cms. from the starting line.

The whole paper was then divided up into 100 equal segments (in accordance to Rf. values) and assayed with oat mesocotyl section test. The results have been presented in Fig. 13.u. In the figure those portions of the chromatogram causing a growth of the mesocotyl sections significantly above controls are spotted, each number in each square

Fig.13. (contd.)

u. Two-way chromatogram of water soluble, ether insoluble fraction of ethanol extract. Arrows on the figure indicate the directions of the first and the second runs. (For further explanation see text.)

FIG.13 (contd.)



• acceleration

∘ inhibition

representing a one per cent. in growth above the fiducial limits. The probable positions of spots have been outlined in the figure from subjective grouping of two or more significant segments.

It would appear that the break down process was not due to drying of paper in between the runs. It could be seen that in the first run the three promotors a, b and c were separated. The position of b extends from .4 - .7 Rf. and possibly the acceleration at this zone was due to two substances P2 and P3 (of Fig. 13.b) which occur in very close Rfs. Substance a (comparable to P1 of Fig. 13.b) has separated out into d, part of g and h. Substance b also showed break-down into e, g and part of i. The long extending zones of g and i were suggestive of fusion of similar substances coming at same Rf. position. It was possible that break-down products of c (comparable to P4 of Fig. 13.b) were f, part of g and part of i.

No colour reactions could be obtained by spraying Ferric chloride/Perchloric acid reagent to chromatograms loaded with alcohol extracts of these water soluble compounds. This observation immediately suggest that indole nuclei are possibly absent in these substances. But, from the results of Audus and Gunning (1958), one cannot be absolutely certain about the chemical nature of these compounds.

(b) Investigations on possible break-down of acid auxins

In view of complex interconversion and break-down processes observed in the water soluble fraction, it was thought that a similar investigation on acid fraction was necessary. In a recent paper Hemberg (1958) has reported that neutral auxins obtained from maize kernels also show break-down phenomena. If such processes really exist in the acid auxins the whole issue of auxin regulated growth in plants has to be reconsidered and modified.

The problem was approached in two ways, viz. (i) colorimetric method and (ii) bioassay method.

In the first stage, two naturally occurring auxins, IAA and IAN, and one precursor TTP were chosen to study their behaviour towards elution and rechromatography. From repeated elution and rechromatography of these substances in isobutanol : methanol : water, no indications of break-down of these compounds were observed. Clear separation of all three substances on a two dimension was also obtained.

It was concluded from these observations that IAA, IAN and TTP are stable compounds under the standard chromatographic procedures followed in this investigation.

To further verify the stability of IAA on elution with alcohol from paper the following experiment was done. The

maximum absorbency of the colour produced by Salper reagent (8 ml.) with IAA (2 ml. of 2 x 10^{-5} g/ml.) was determined with a Unicam spectrophotometer. Alcoholic solution of IAA was spotted on paper, run in solvent and the IAA zone of the paper was eluted with alcohol. The alcohol was evaporated off and again taken up in 2 ml. of alcohol. Salper reagent (8 ml.) was added to it and the maximum absorbency was determined after 25 minutes. The maximum absorbency was obtained in both cases at 530 mμ. This was also an indirect evidence for stability of IAA.

In the next stage duplicate chromatograms were prepared from the acid-ether fraction of TTP-incubated root extracts. About 10 gms. of 7 days' old tissue was used in each experiment. One of them was sprayed with Ehrlich's reagent and three well-defined spots could be seen. Corresponding zones were cut off from the second chromatogram, eluted in alcohol and rechromatographed. The chromatograms were developed and sprayed for colour reaction. The following table shows the result:-

Ehrlich positive zones on 1st chromatogram marker. Rf.	Rf. .1-.2 of 2nd chromatogram eluted and rechromatographed. Rf.	Rf. .42-.55 of 2nd chromatogram eluted & rechromatographed. Rf.	Rf. .7-.8 of 2nd chromatogram eluted and rechromatographed. Rf.
.1 - .2	0 - .15	-	-
.42-.55	-	.38-.5	-
.7 - .8	-	-	.6 - .75

Fig.13. (contd.)

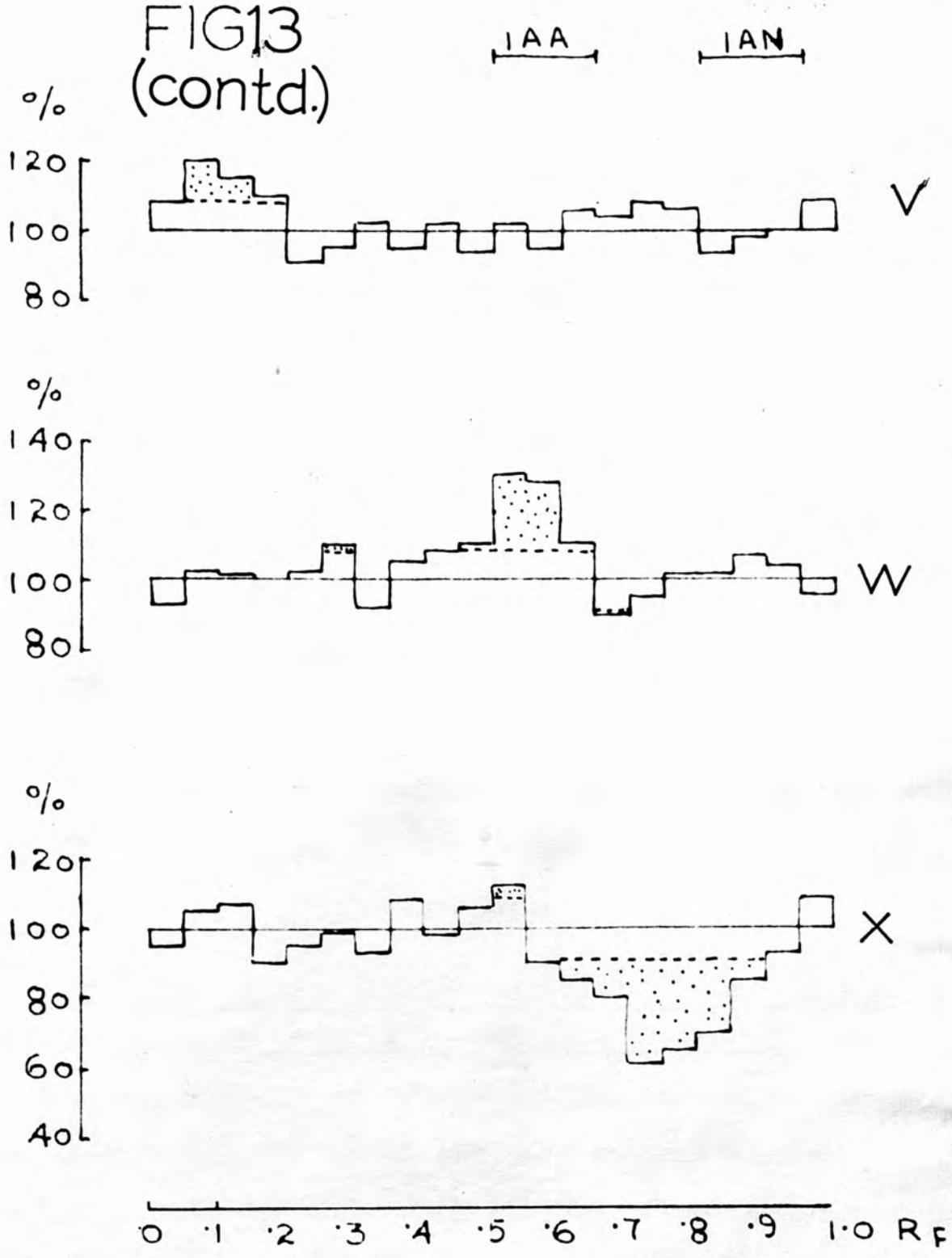
Chromatograms from the eluates of a primary chromatogram of the acid ether fraction of an ethanol extract. Oat coleoptile assay was used for assay of chromatogram. (Growth responses plotted against Rf. values.)

v. Chromatogram obtained from elution and rechromatography of the accelerator α zone of the primary chromatogram.

w. Chromatogram obtained from elution and rechromatography of the IAA zone of primary chromatogram.

x. Chromatogram obtained from elution and rechromatography of the inhibitor β zone of the primary chromatogram.

FIG13 (contd.)


IAA
IAN
%
120
100
80
V
%
140
120
100
80
W
%
120
100
80
60
40
X
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

From these experiments it was confirmed that indole compounds that are formed by incubation with TTP do not break down due to elution and chromatography.

In the next experiment the stability of endogenous acid auxins was studied by bioassay method.

Duplicate chromatograms of acid fraction were prepared from two extracts, each containing 35 gms. of 7 days' old roots of broad bean. One of them (Table 13, Appendix) was assayed to show the growth active zones. The corresponding zones from the second chromatogram were eluted in alcohol, rechromatographed and assayed with oat coleoptile sections. The resulting histograms are presented in Fig.13 (v, w and x). It would appear from the results that accelerator α, IAA and inhibitor β, do not show any interconversion or breakdown due to elution and rechromatography.

c. Investigations on the fate of certain synthetic growth substances and precursors in the root tissue of broad bean

Metabolic sequence of events leading to biogenesis of natural root auxins is one of the many enigmas of this field of research. Considerable work has been done in relation to other parts of plants, but there are very few papers devoted to the study of roots.

The current concepts of IAA formation in the plant tissue are discussed by Larsen (1952), Gordon (1954, 1955) and others.

On the basis of works of Thimann (1935), Skoog (1937) and Wildman, Ferri and Bonner (1947) and others, a general picture regarding the synthesis of IAA in plant tissues has been formulated. The general course of reaction arising from TTP through IPyA or TNH_2 and IAc may not be operating in all tissues. An alternative path through IAN and IAM has also been suggested (Jones, Henbest, Smith and Bentley, 1952) and demonstrated (Stowe and Thimann, 1954), (Mahuderan, Sundararman and Thimann, 1958). Dannenburg and Liverman (1957), however, suggested from their investigation on conversion of TTP-$2c^{14}$ in watermelon tissue, that IPyA is an intermediate in TTP to IAA reaction. They also observed that when the pathway through IAc is blocked, TNH_2 and IAN are formed from TTP.

On the basis of these findings, the present investigation was carried out to obtain some information about the enzyme regulated transformations of certain synthetic growth substances and precursors that occur within the root tissue.

In the preliminary experiment the distribution pattern of IAA, IAN and TTP was determined in isobutanol : methanol : water solvent system. Known amounts of these substances were taken up in 35 ml. of aqueous solution and it was fractionated. The acid ether, neutral ether and water soluble parts were separately chromatographed. The distribution of these

substances in different fractions are shown in the following table:

Table 8.

Substance	Acid Fr. Rf.	Neutral Fr. Rf.	Water soluble Fr. Rf.	Colour with Ehrlich's reagent
TTP	-	-	0 - 0.1	Pinkish violet
IAA	0.45-0.67	-	-	" "
IAN	0.8 -0.95 **	0.8-0.95 ****	" -	Greenish yellow

It was observed that IAN can occur in both acid and neutral ether fractions.

In the next stage broad bean root tissues (obtained from 9-10 days' old plants) were fed with TTP, IAA, IAN and TNH_2 and the fractions were separately analysed. (Method of feeding has been previously described.) The results are presented in the following table:

Table 9.

Solvent - Isobutanol : Methanol : water (80 : 5 : 15)

Compounds incubated with, or known compounds	Fraction	Rf.values	Colour with Ehrlich's reagent	Colour intensity	Probable compound
TTP (1st Expt.)	Acid	0 - 0.1	Pinkish-violet	*	IAAP?
		0.52-0.63	"	***	IAA
		0.7 - 0.8	"	**	
		0.85-0.95	Gray	diffused	
	Neutral	0 - 0.5	Pinkish-violet	*	
	Water sol.	0 - 0.06	"	****	TTP

(contd.)

Table 9 (contd.)

Compounds incubated with, or known compounds	Fraction	Rf.values	Colour with Ehrlich's reagent	Colour intensity	Probable compound
TTP (2nd Expt.)	Acid	0.14-0.2	Pinkish-violet	**	IAAP
		0.38-0.5	"	***	IAA
		0.67-0.79	"	**	
		0.8 -0.95	Gray	diffused	
	Neutral	-	-	-	
	Water sol.	0 -0.05	Pinkish-violet	****	TPP
IAA	Acid	0 -0.15	Pinkish-violet	**	IAAP?
		0.45-0.77	"	****	IAA
		0.8 -0.85	Light pink	*	
	Neutral	0.55-0.75	" "	*	
IAN	Acid	0.17-0.25	Bluish gray	***	
		0.32-0.35	" green	**	
		0.45-0.49	Deep yellow	***	
		0.8 -0.95	Pinkish-violet	**	
	Neutral	0.65-0.9	Deep greenish yellow (tailing)	****	
IAN (previous chromatogram - colour observed after 24 hours)	Acid	0.6 -0.65	Pink	*	
		0.7 - 0.75	Pinkish-violet	*	
		0.8 -0.85	Greenish-yellow	****	
		0.85-0.95	Pink	**	

(contd.)

Table 9 (contd.)

Compounds incubated with, or known compounds	Fraction	Rf.values	Colour with Ehrlich's reagent	Colour intensity	Probable compound
TNH_2	Acid	0 -0.15	Pinkish-violet	*	
		0.86-1.0	"	*	
	Neutral	0.3 -0.4	"		
		0.7 -0.85	" turning bluish-green		
IAA		0.38-0.55			
IAN		0.8 -0.95			
IBA		0.1 -0.25			
TNH_2		0.1- 0.27			
TTP		0 - 0.1			
IPrA		0.6 -0.8			

It would appear from these observations that TTP was converted into IAA and three other acid ~~auxins~~ Ehrlich positive substances due to enzymatic reaction. But with TNH_2 no colour reactions were observed in the IAA region of the chromatogram. With IAA, three indole compounds were formed and the one at low Rf. value was possibly IAAP.

IAN produced a large number of acid-ether soluble compounds, but none of these could be related to IAA on the

basis of colour reaction. IAN to IAA transforming enzymes seem to be absent in broad bean root tissue.

It was previously noted that the substance at low Rf. value (around Rf. 0-0.1) obtained by TTP feeding does not show any break down products on chromatography. In the same solvent system when IPyA was chromatographed four decomposition products were observed after spraying with Ehrlich's reagent. Their positions on chromatogram are shown in the following table:

Table 10.

Rf.	Colour by Ehrlich's reagent		Known compound with same Rf.
0.05-0.17	Pinkish violet turning yellow	**	
0.15-0.4	Greenish yellow	****	
0.45-0.6	Pinkish violet	***	IAA
0.65-0.8	Greenish yellow	**	

The results indicate certain interesting facts about the metabolism of growth substances in broad bean roots. Firstly, if IAA is found to be a naturally occurring growth substance in this tissue, t e possible precursor would be TTP and not TNH_2. Secondly, IAN to IAA transforming enzyme system is in all probability absent in this tissue. Thirdly, the substance that is formed at Rf. values lower than IAA due to TTP or IAA

feeding is possibly IAAP and not IPyA. Because it has been seen before that this substance is stable towards elution and rechromatography in isobutanol : methanol : water solvent system, whereas synthetic IPyA produced four Ehrlich's positive substances when chromatographed in the same neutral solvent.

3. Final investigations (following fractionation method)

In the final investigations on root hormones, purification and separation of growth substances were done according to the methods followed by Bennet-Clark and Kefford (1952), Kefford (1955), and Audus and Thresh (1956). It has now been established that this method is, at least, quantitative for IAA. Since in a crude extract several forms of both growth-promoting and growth-inhibiting substances may be present, a fractionation of the extract should be done before testing them for biological activity. Moreover, different fractions (acid, neutral and water soluble) could be investigated without interference of one another and some information could be gathered about the nature of the compounds.

In all experiments, from here onwards, broad bean roots have been used. Special emphasis has been given to the analysis of acid fraction extracts, but neutral and water soluble parts were also tested for growth activity, from time to time.

(a) Auxin content of broad bean roots in relation to the age of the seedlings

The relationship of ageing and endogenous balance of auxins is rather enigmatic. The statement that the young roots have suboptimal auxin which gradually increases with age and becomes supraoptimal, has not yet been established. In the first part of the investigation auxin content of the whole root has been chromatographically analysed at different stages of growth. Later, the investigation was directed towards a chromatographic study of auxins in different parts of root system at different ages of seedlings.

(i) Auxins in the acid fraction

In Figs. 14-21, results of bioassays of chromatograms of acid fractions of root extracts obtained from different ages of seedlings have been illustrated as histograms. Oat mesocotyl assay was used in these experiments. The results of experiments performed on different occasions with root extracts from same ages of plants have been presented as separate histograms under each figure.

In three days' old roots (Fig.14, a, b and c), four promotors and one inhibitor could be detected. The regions of stimulations were around 0.1, 0.25, 0.5 and 0.9, and significant inhibition was recorded in two experiments in regions just after the IAA zone.

In these, as also in later experiments, it was observed that oat mesocotyl assay shows a poor response to inhibitors. This is in support of Bentley's (1958) observation. Significant inhibitions due to inhibitor were recorded on rare occasions in this set of results.

The results of analysis of root extracts from 4, 9, 12, 15, 18, 22 and 25 days' old plants are shown in Figs.15-21. In most of these histograms three growth promotors could be observed. They were around 0.1 (accelerator α), 0.45 (IAA position) and 0.9 Rf. values. The promotor at 0.25 Rf. value detected in 3 days' old root was, however, absent in older roots.

It is worth mentioning that Rf. values of active spots, as well as that of marker IAA and IAN shifted slightly in different experiments. Nevertheless, the relative position of growth active zones remained fairly constant.

The picture of endogenous acid auxins as observed in these experiments is comparable to that observed by Kefford (1955) in the same material. Following the oat coleoptile section test, the previous author (Kefford, 1955) could demonstrate the presence of accelerator α, IAA and inhibitor β in broad bean roots. He could not show the promotor at higher

Rf. value (about 0.9). But this substance has been found to be highly active in mesocotyl assay and was present in almost all extracts. For convenience this substance will be referred to as accelerator x in later discourse.

It can be seen that each experiment has been repeated several times with a view to obtaining a quantitative picture of endogenous auxins at different ages of roots. The quantitative relationship of endogenous auxins and ageing that has been revealed by these experiments has certain physiological interest. What is really wanted, of course, is the changes in the cell ages. The data that have been presented here are not very helpful in that respect. Nevertheless, a direct relationship between the auxin content of the whole root system and the age of the seedling can be deduced from these data.

In order to do this, activity of each of the growth promotors in each histogram was determined in IAA-equivalents from the standard calibration curve. (Table 5, Appendix) Results of all the experiments were further summed up in Figs. 22 and 23 where concentrations in µgm/hgm. IAA eq. (mean values) of total acid auxin, IAA, accelerator and accelerator x are shown as functions of age of seedlings.

These results directly show that in broad bean roots endogenous auxins fall in concentrations as age of the seedling increases. These changes are very remarkable. In 3 days' old roots 0.728 µgm/hgm. of total acid auxins was recorded which went up to 1.82 µgm/hgm. on the 9th day. From the 12th day (1.76 µgm/hgm.) onwards the concentration gradually started to fall. The substance occurring at IAA zone presented an identical picture. From 0.49 µgm/hgm. on the 3rd day, it increased to 1.755 µgm/hgm. on the 9th day and 1.55 µgm/hgm. on the 12th day. But on the 15th day 0.45 µgm/hgm. was observed and the concentration gradually came down to a very low value (0.006 µgm/hgm.) on the 25th day.

Accelerator α and accelerator x showed rather irregular shifting of concentrations from 3rd to 12th day, but later gradual fall is noticeable in both cases. Concentration changes of inhibitor β could not be detected because experiments were carried out with oat mesocotyl section test.

It is interesting to note the growth of roots at the same time. In Fig. 24 mean lengths of roots and shoots have been plotted against age of seedlings. Similarly, weights of tap roots, laterals and shoots at different ages are shown in Fig. 25. It would appear from the graphs that root growth

ceased completely after 12-15 days, but shoots showed indications of unrestricted growth. It is interesting to note again that after 12-15 days root auxins also fall in concentration.

It may be observed that quantities of growth substances recorded in Table 3 (Appendix) show considerable variations in similar experiments performed on different occasions. Such variability was unavoidable even under careful experimental manipulations. The variations might be due to three primary causes: (a) biological variation in the assay material, (b) variability in auxin content in plants from which extracts were prepared, and (c) variations in extraction losses.

The second cause has been found to be true. In one experiment 3 days' old seedlings were selected out in different groups according to their root lengths and their growths were measured at different lengths of tims. It was observed that each group showed different rates of growth (Table 7, Appendix). It would be logical to deduce that, although the physiological age of seeds were the same, the physiological balance of auxins would be different in each group of plants. Hence, fluctuations in auxin contents in the ultimate assay must occur on different occasions. However, it may be mentioned that in all experiments random samples were collected

for extraction purpose. Some workers have worked out ageing problems and auxin content of roots on the basis of selected samples showing equal root lengths. But it must be emphasised that age of a plant cannot be related to its root length and can never be used as a standard in investigations on ageing. The true age of a plant is definitely related to the germination of the embryo. No matter what the root lengths are, plants germinating at the same time, after being subjected to equal treatment of soaking and sowing, all belong to the same age group.

(ii) Neutral fractions

The neutral ether fractions obtained from previous set of experiments were also chromatographically tested for growth activity.

When the chromatography was carried out in Isobutanol : Methanol : water, results were very inconsistent. This was thought to be due to ether soluble impurities which interfered with bioassay. However, when water was used as solvent the impurities remained immobile at the starting point. In most of the chromatograms where water was used as a solvent, a promotor of oat mesocotyl sections was noted at low Rf. values (about 0.1 Rf.). However, no definite conclusions could be deduced from these results (Table 6, Appendix), because the results were not reproduceable.

Fig.14. Oat mesocotyl assay of chromatograms of acid fractions of root extracts obtained from 3 days' old broad bean plants. The histograms (a), (b) and (c) illustrate results obtained on three different occasions.

a. 40.0 gm. of tissue used for extraction.
b. 16.5 gm. of tissue used for extraction.
c. 30.0 gm. of tissue used for extraction.

FIG 14

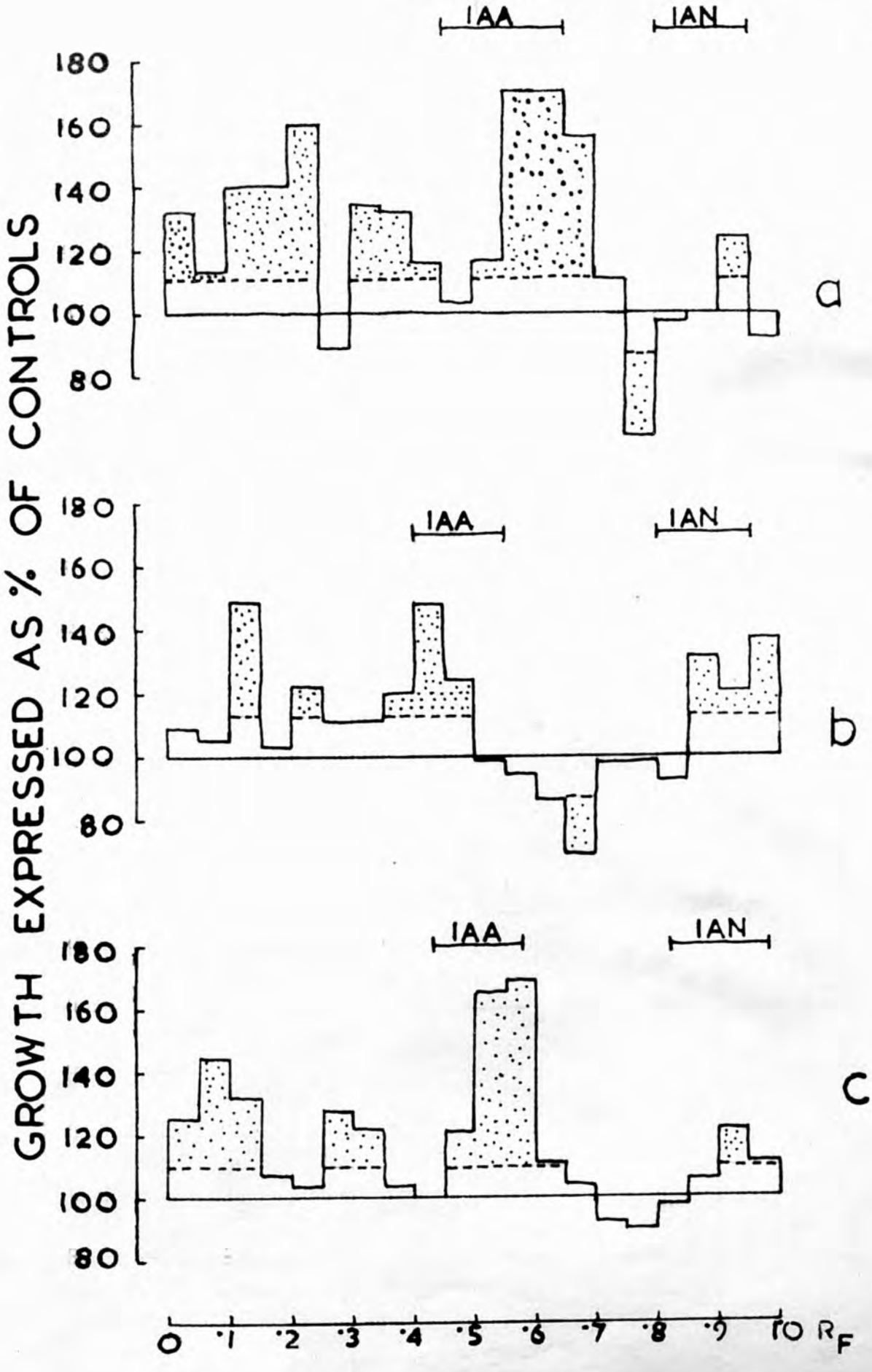

IAA
IAN
3 DAYS
180
160
140
120
100
80
GROWTH EXPRESSED AS % OF CONTROLS
a
b
c
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 R_F

Fig.15. oat mesocotyl [redacted] assay of the chromatogram of the acid fraction of root extracts obtained from 4 days' old broad bean roots.

a. 40.0 gm. of tissue used for extraction.

FIG15

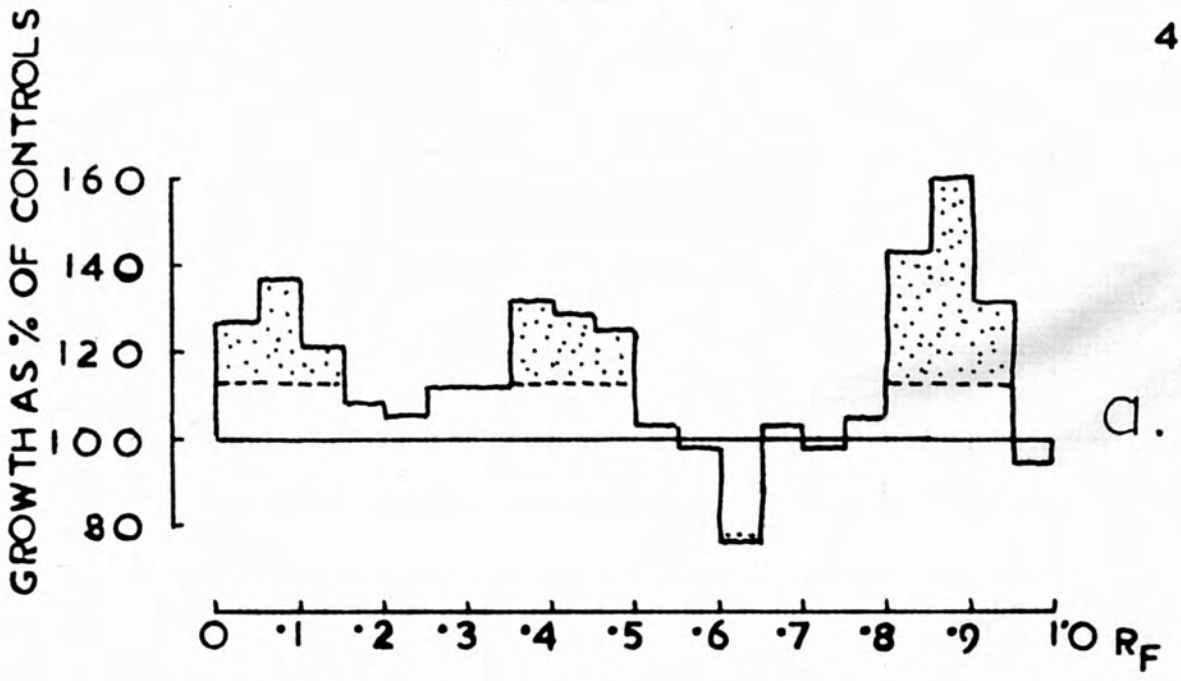

4 DAYS
GROWTH AS % OF CONTROLS
160
140
120
100
80
a.
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Fig.16. Oat mesocotyl assay of chromatograms of acid fractions of root extracts obtained from 9 days' old broad bean roots. The histograms (a), (b) and (c) illustrate results obtained on three different occasions.

a. 40.0 gm. of tissue used for extraction.
b. 30.0 gm. of tissue used for extraction.
c. 40.0 gm. of tissue used for extraction.

FIG 16

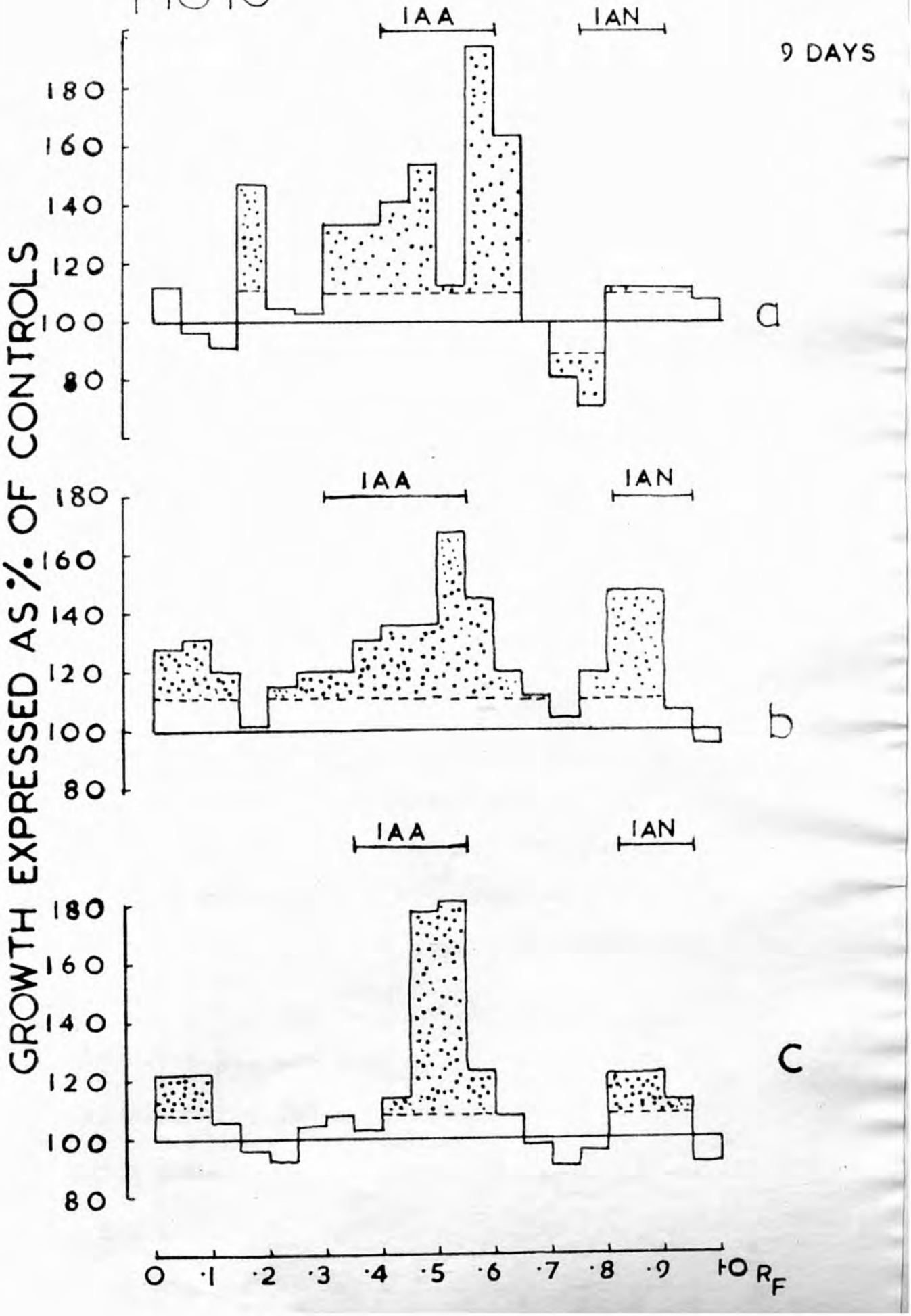

IAA
IAN
9 DAYS
180
160
140
120
100
80
GROWTH EXPRESSED AS % OF CONTROLS
a
b
c
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 R_F

Fig.17. Oat mesocotyl assay of chromatograms of acid fractions of root extracts obtained from 12 days' old broad bean plants. The histograms (a), (b) and (c) illustrate results obtained on three different occasions.

a. 35.0 gm. of tissue used for extraction.
b. 30.0 gm. of tissue used for extraction.
c. 40.0 gm. of tissue used for extraction.

FIG17

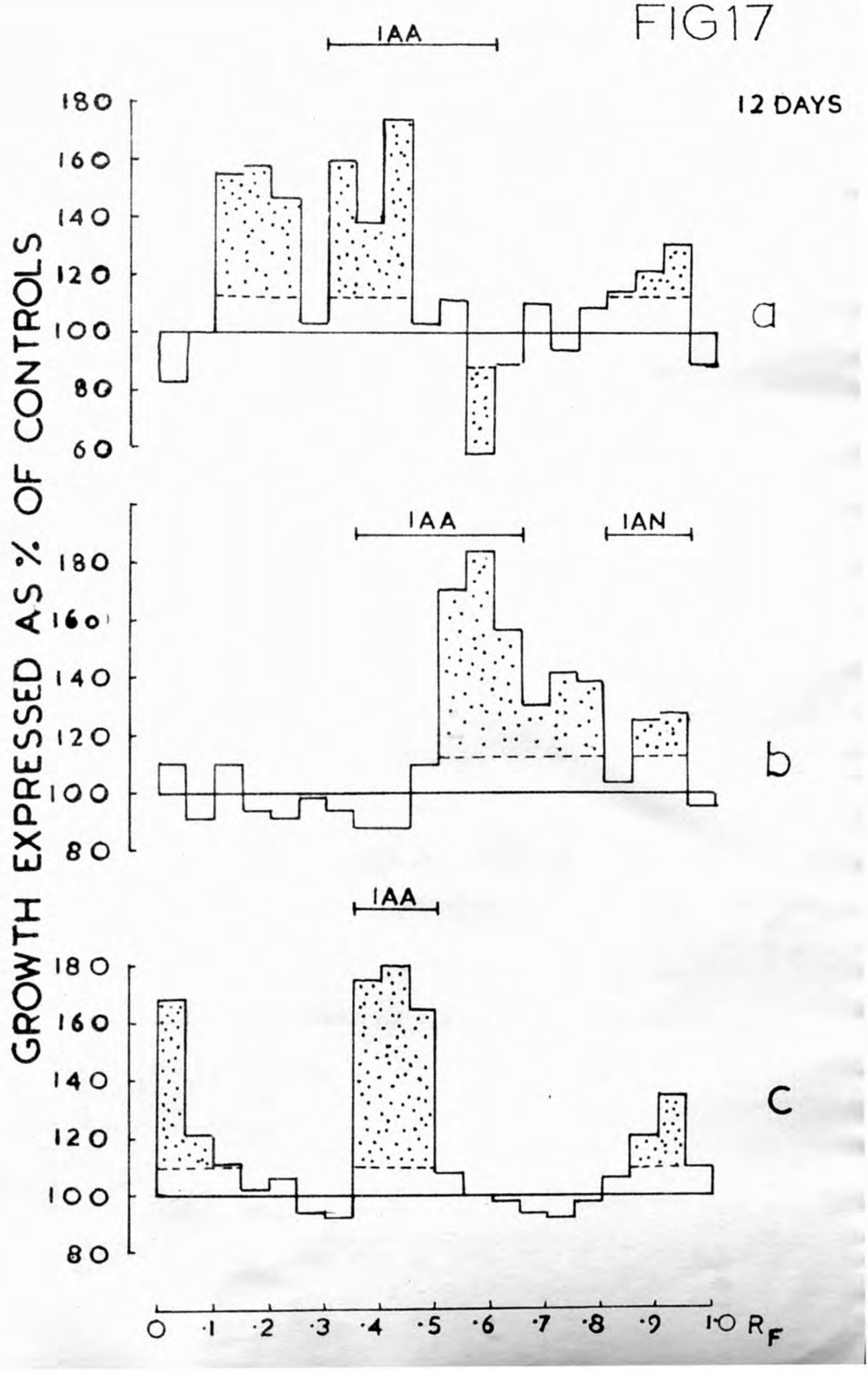

IAA
12 DAYS
180
160
140
120
100
80
60
a
IAA
IAN
180
160
140
120
100
80
b
IAA
180
160
140
120
100
80
c
GROWTH EXPRESSED AS % OF CONTROLS
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 R_F

Fig.18. Oat mesocotyl assay of chromatograms of acid fractions of root extracts obtained from 15 days' old broad bean plants. The histograms (a), (b) and (c) illustrate results obtained on three different occasions.

a. 40.0 gm. of tissue used for extraction.
b. 34.0 gm. of tissue used for extraction.
c. 40.0 gm. of tissue used for extraction.

FIG.18.

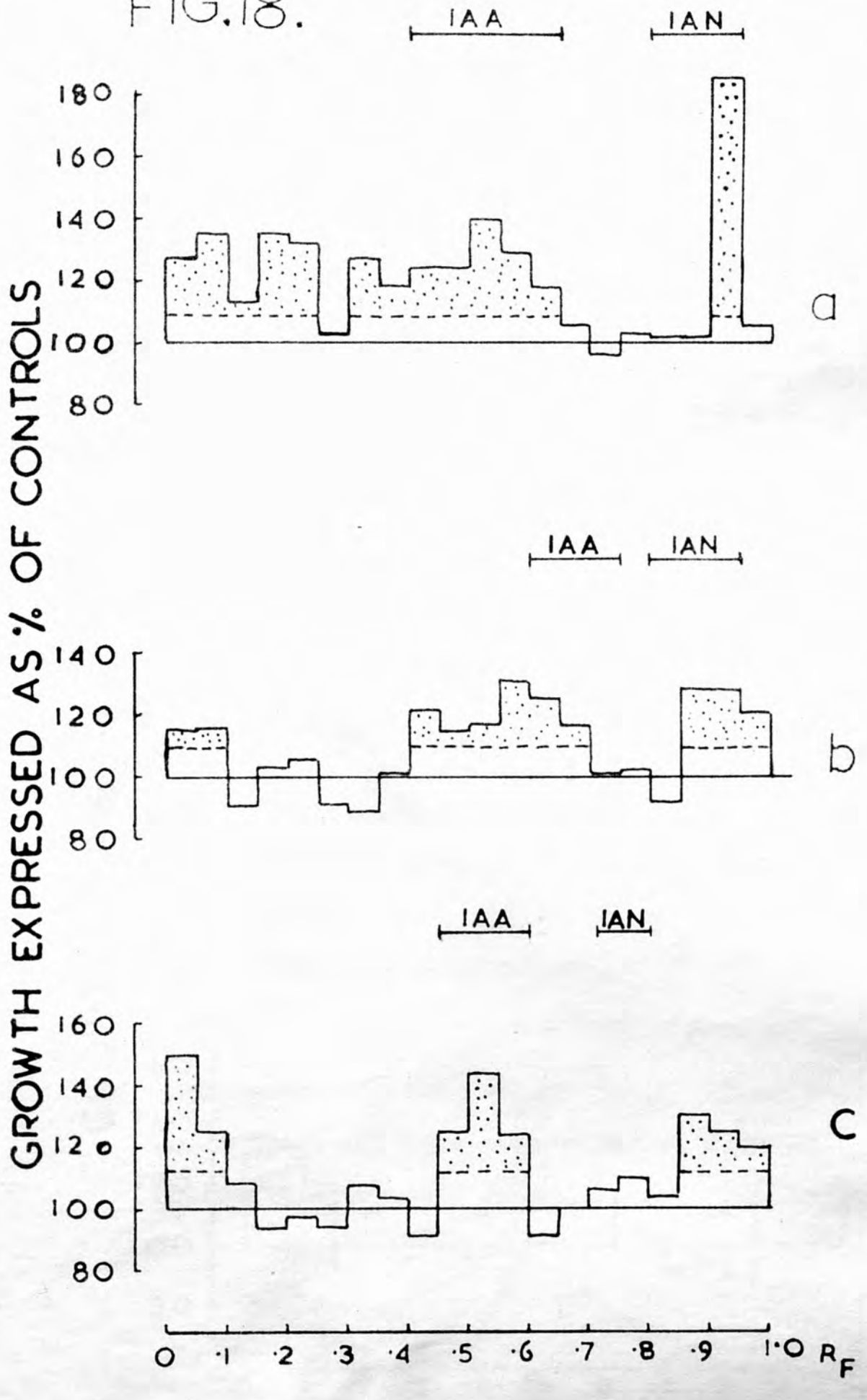

IAA
IAN
15 DAYS
180
160
140
120
100
80
a
IAA
IAN
140
120
100
80
b
IAA
IAN
160
140
120
100
80
C
GROWTH EXPRESSED AS % OF CONTROLS
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Fig.19. Oat mesocotyl assay of chromatograms of acid fractions of root extracts obtained from 18 days' old broad bean plants. The histograms (a), (b) and (c) illustrate results obtained on three different occasions.

a. 40.0 gm. of tissue used for extraction.
b. 40.0 gm. of tissue used for extraction.
c. 30.0 gm. of tissue used for extraction.

FIG. 19.

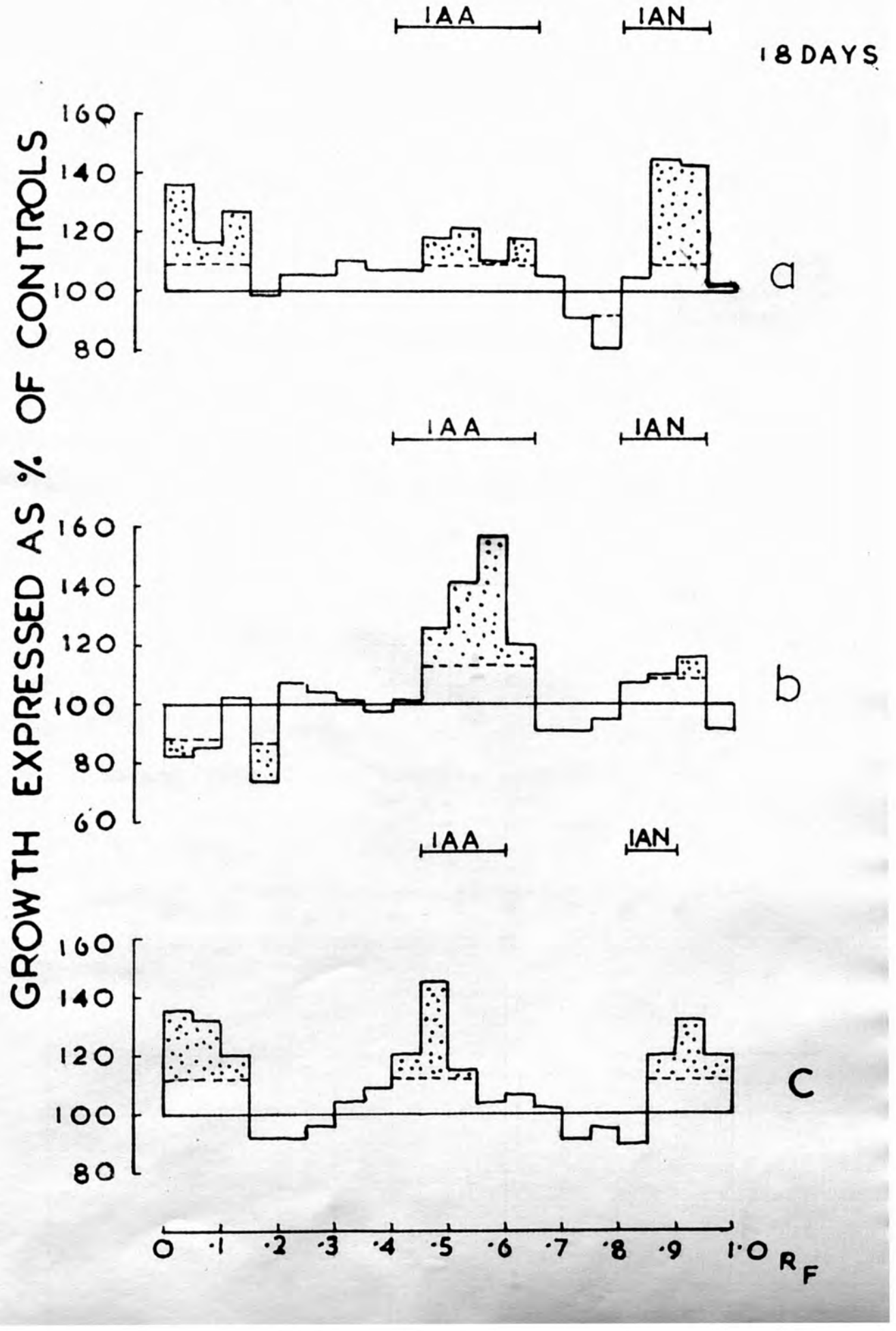

IAA
IAN
18 DAYS
GROWTH EXPRESSED AS % OF CONTROLS
160
140
120
100
80
a
IAA
IAN
160
140
120
100
80
60
b
IAA
IAN
160
140
120
100
80
C
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Fig.20. Oat mesocotyl assay of chromatograms of acid fraction of root extracts obtained from 22 days' old broad bean plants. The histograms (a) and (b) illustrate results obtained on two different occasions.

a. 30.0 gm. of tissue used for extraction.

b. 30.0 gm. of tissue used for extraction.

FIG. 20.

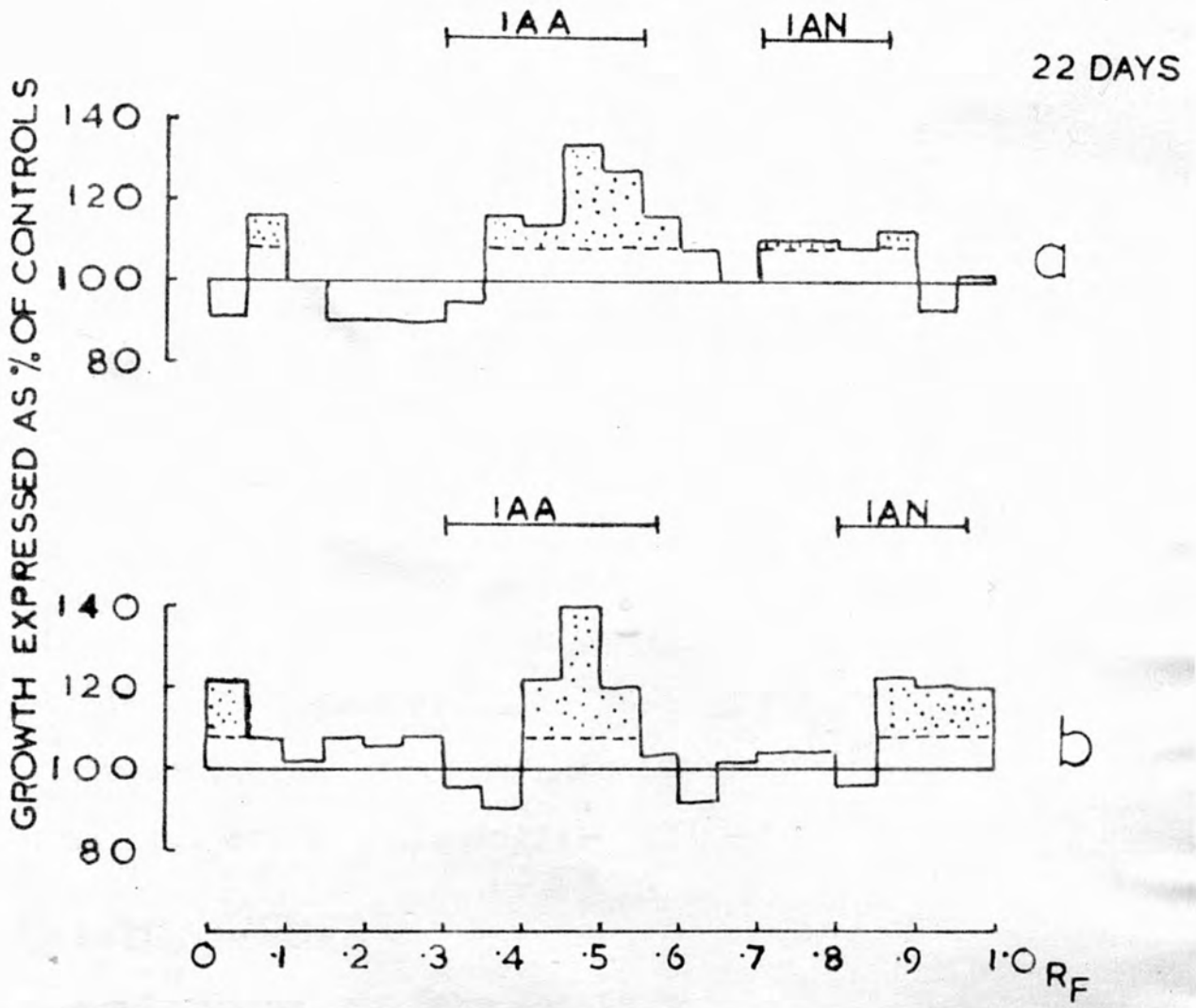

IAA
IAN
22 DAYS
GROWTH EXPRESSED AS % OF CONTROLS
140
120
100
80
a
IAA
IAN
140
120
100
80
b
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Fig.21. Oat mesocotyl assay of chromatograms of acid fractions of root extracts obtained from 25 days' old broad bean roots. The histograms (a), (b) and (c) illustrate results obtained on three different occasions.

a. 30.0 gm. of tissue used for extraction.
b. 40.0 gm. of tissue used for extraction.
c. 40.0 gm. of tissue used for extraction.

FIG. 21.

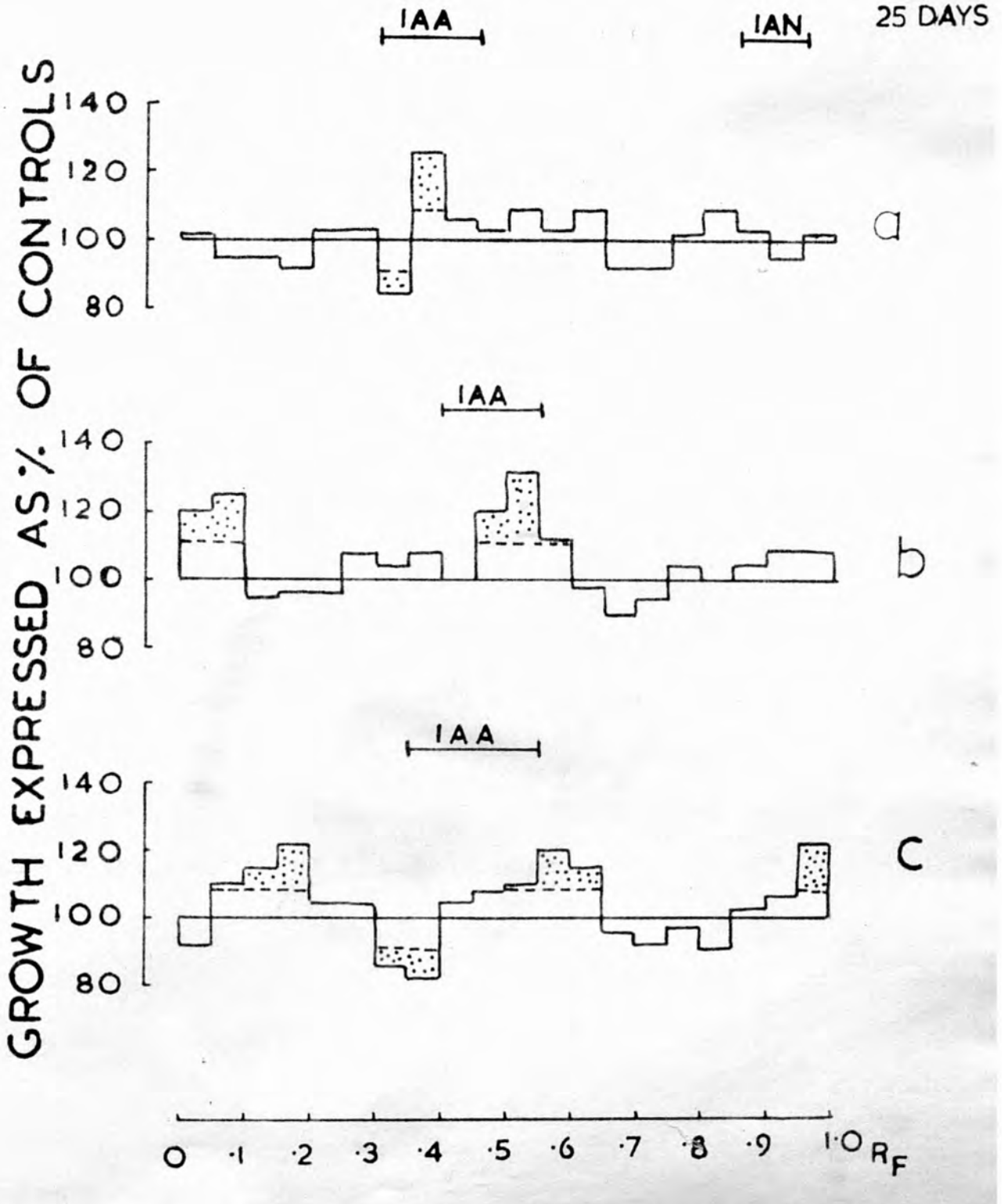

IAA
IAN
25 DAYS
GROWTH EXPRESSED AS % OF CONTROLS
140
120
100
80
a
IAA
140
120
100
80
b
IAA
140
120
100
80
C
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Fig.22. Quantitative changes in the amounts of IAA (i.e. substance occurring at the IAA region of chromatograms) and total acid auxins in the broad bean root tissue at different ages of the seedlings. Abscissae: Age of seedlings in days, starting from the date of sowing. Ordinates: Amounts of IAA or total acid auxin in μgm/hgm IAA equivalents.

FIG. 22.

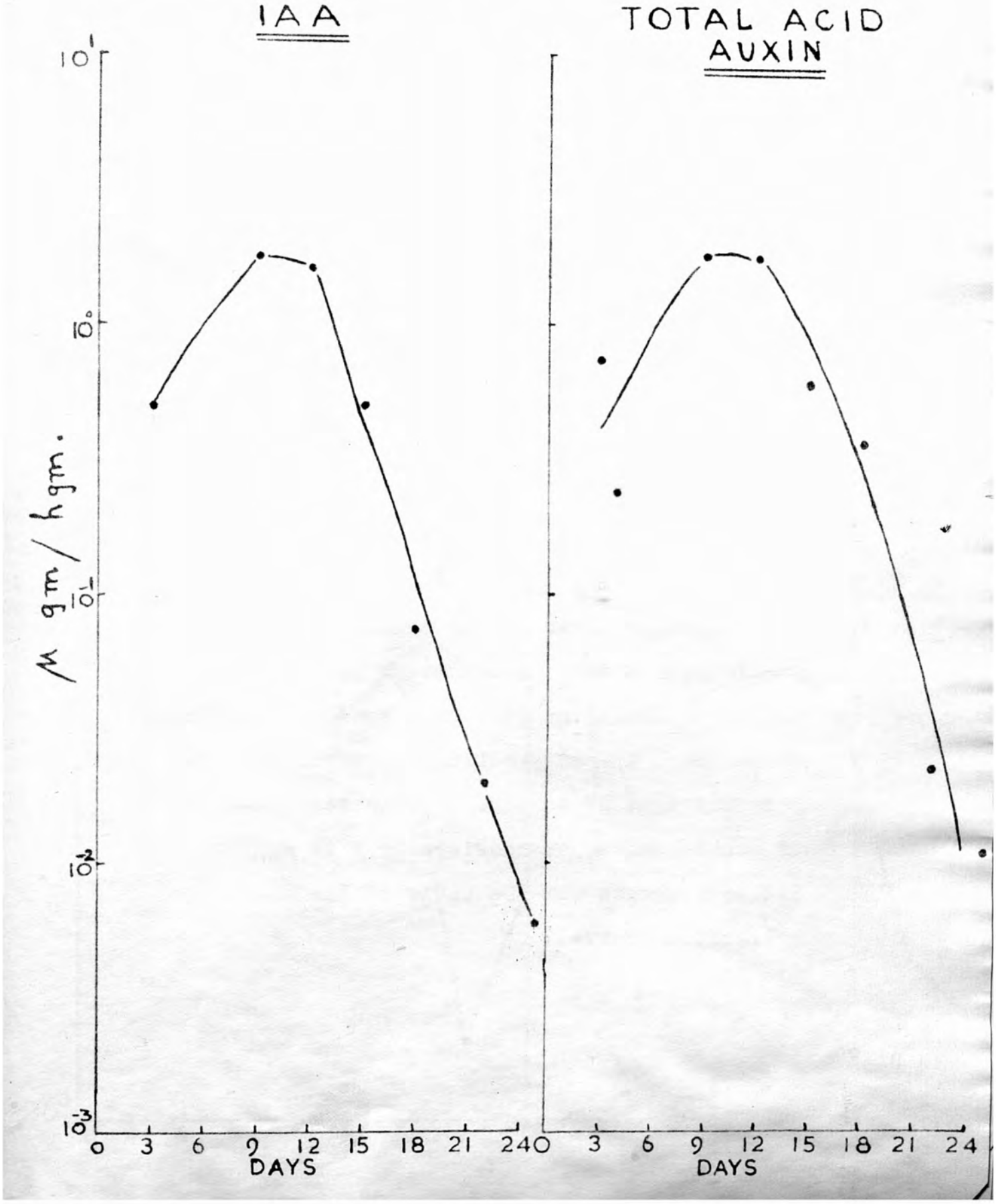

IAA
TOTAL ACID AUXIN
10¹
10⁰
10⁻¹
10⁻²
10⁻³
M gm / kgm.
0 3 6 9 12 15 18 21 24
DAYS
0 3 6 9 12 15 18 21 24
DAYS

Fig.23. Quantitative changes in the amounts of accelerator ∝ and accelerator x in the broad bean root tissue at different ages of seedlings. Abscissae: Age of seedlings in days, starting from the date of sowing. Ordinates: Amounts of accelerator ∝ or accelerator x in γgm/hgm IAA equivalents (on the basis of IAA calibration curve).

FIG. 23.

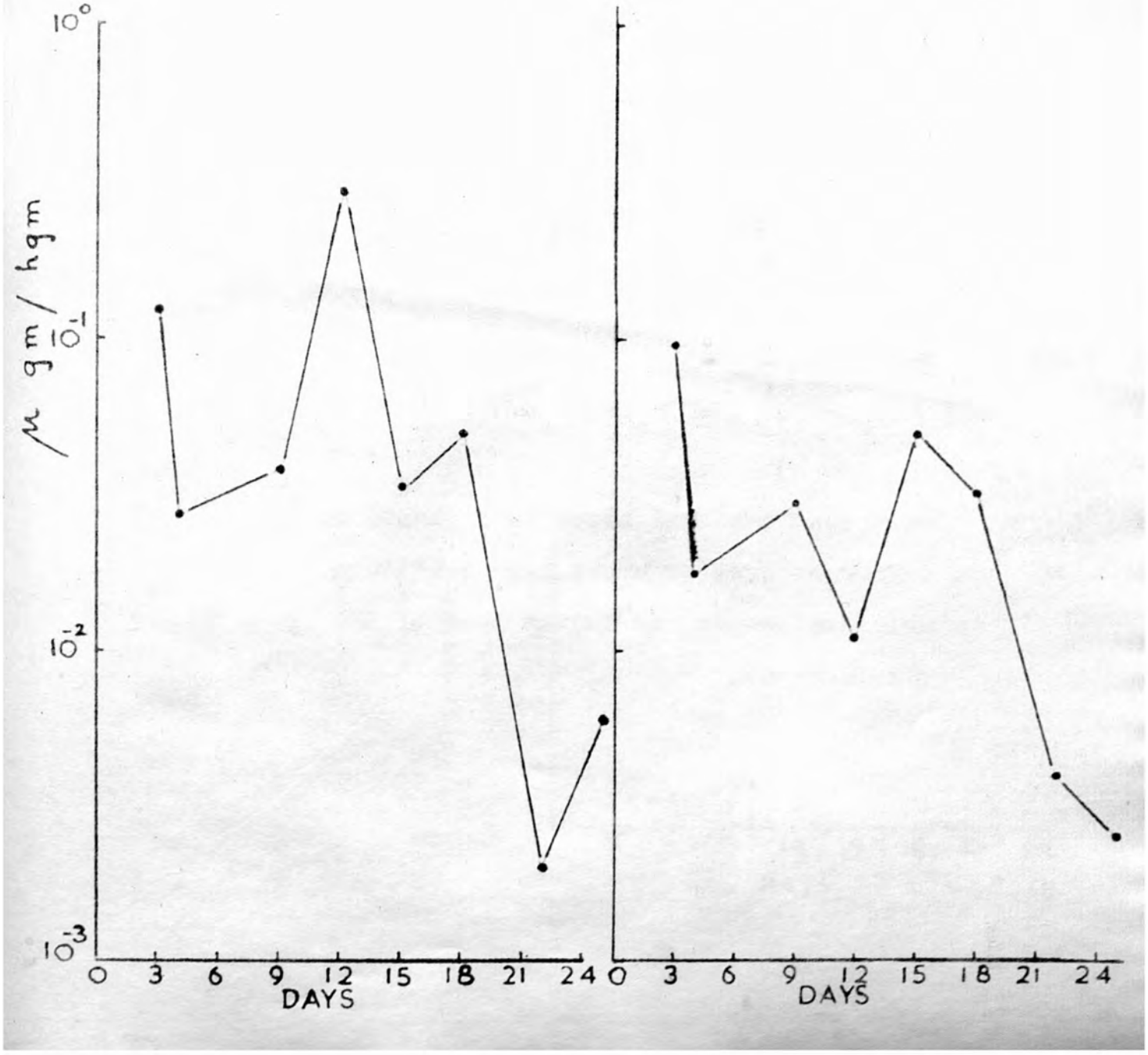
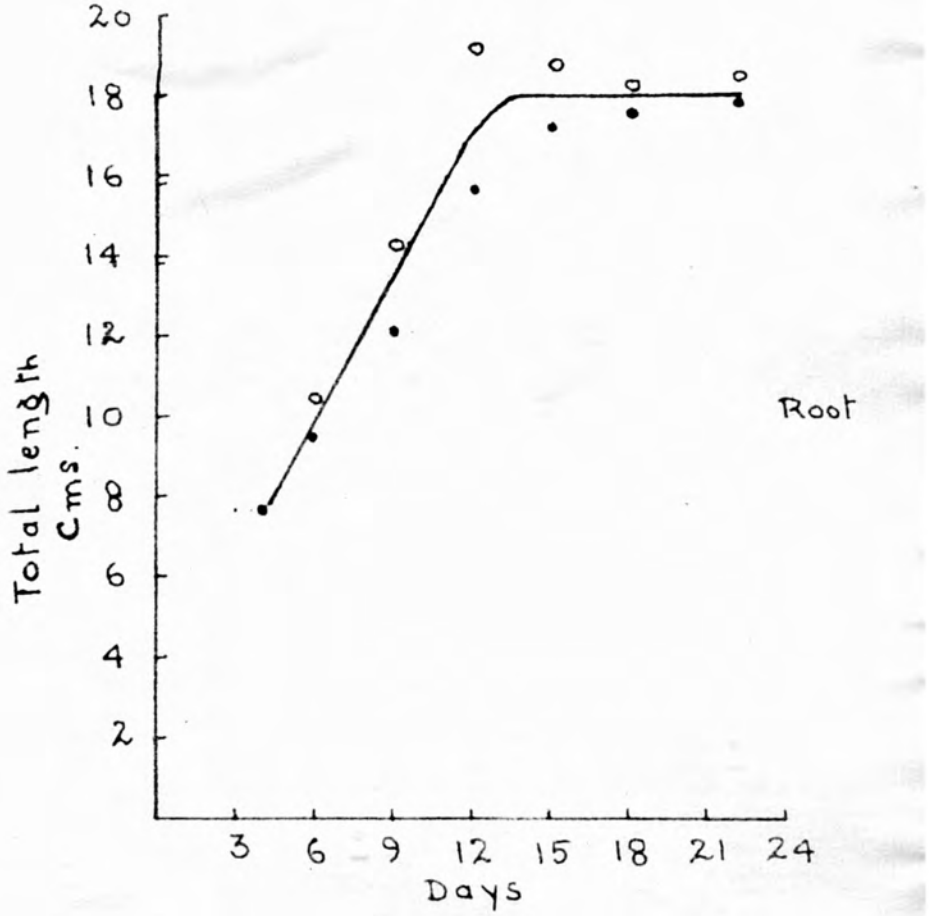

ACCELERATOR 'α'
ACCELERATOR 'X'
10°
10⁻¹
10⁻²
10⁻³
μ gm / kgm
0 3 6 9 12 15 18 21 24
DAYS
0 3 6 9 12 15 18 21 24
DAYS

Fig.24. Lengths of root and shoot (per plant) at different ages of broad bean seedlings. Each point on graphs represents mean of 10 observations.

FIG.24.


Total length Cms.
20
18
16
14
12
10
8
6
4
2
3
6
9
12
15
18
21
24
Days
Root

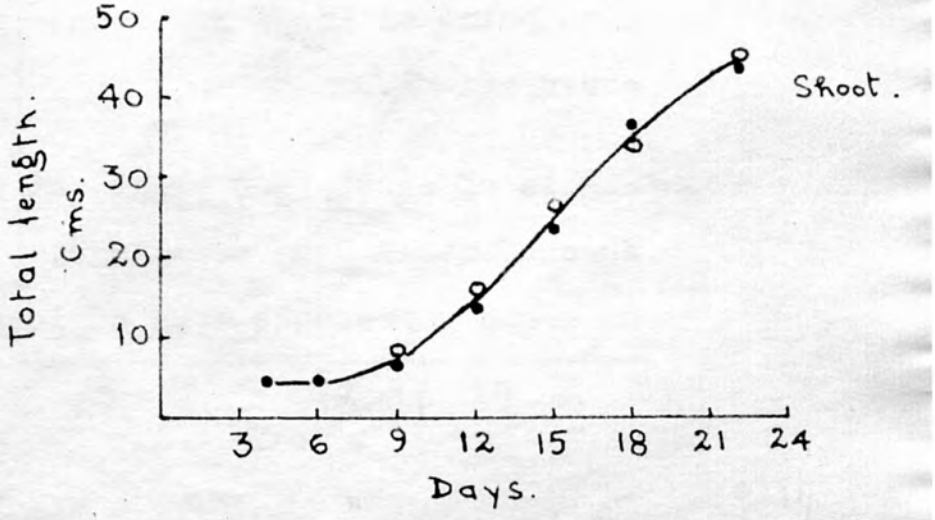
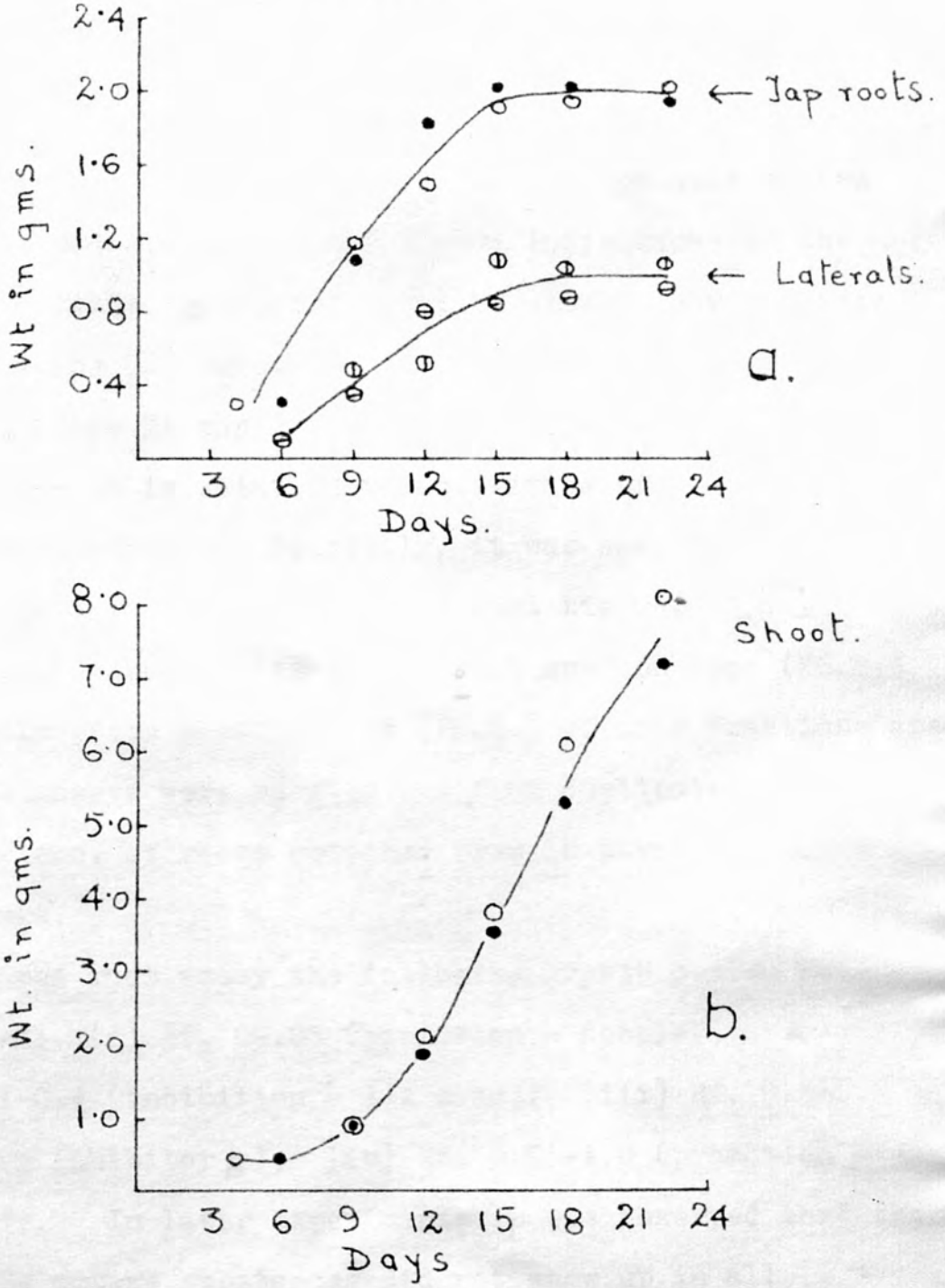

Total length. Cms.
50
40
30
20
10
3
6
9
12
15
18
21
24
Days.
Shoot.

Fig.25. a. Weights of tap root and laterals (per plant) at different ages of broad bean seedlings. Each point on graph represents mean of 10 observations.

b. Weights of shoot (per plant) at different ages of broad bean seedlings. Each point on graph represents mean of 10 observations.

FIG. 25.


2·4
2·0
1·6
1·2
0·8
0·4
Wt in gms.
← Tap roots.
← Laterals.
a.
3 6 9 12 15 18 21 24
Days.
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
Wt. in gms.
Shoot.
b.
3 6 9 12 15 18 21 24
Days

(b) Activity curves of acid growth substances of broad bean roots in different assay methods

It was observed in previous experiments that three promotors (accelerator α, IAA and accelerator x) could be detected by oat mesocotyl section test. In very young roots (3 days' old), a fourth promotor was also found just before IAA region. Besides these, there were indications of the presence of inhibitor β, but mesocotyls showed poor response to this substance (or substances?).

At this stage it was realised that more information should be gathered in relation to activities of these substances to other assay methods. Specially, it was essential to study the effects of these substances on a root tissue.

In Fig. 26, the results of pea root section test (26.a.) and wheat coleoptile section test (26.b.) of acid fractions are shown. The assays were carried out from duplicate extracts of 50 and 40 gms. of roots obtained from 10 days' old broad bean seedlings.

In the pea root assay the following growth active zones were detected: (i) Rf. 0-.05 (promotion - accelerator α); (ii) Rf. 0.1-0.4 (inhibition - IAA zone); (iii) Rf. 0.5-0.8 (inhibition - inhibitor β); (iv) Rf. 0.95-1.0 (promotion - substance x?). In later experiments it was observed that the last of these active substances did not show up in all

experiments due to possible interference with inhibitor β. In wheat coleoptile test accelerator α and IAA produced promotion of growth and inhibitor β produced inhibition. No trace of growth activity was observed after inhibitor β zone. Absence of promotor x in wheat coleoptile assay could again be due to masking effect of inhibitor β. A slight shifting of Rf. value of inhibitor β zone is noticeable in the wheat coleoptile test. When the neutral fraction was chromatographed in Isobutanol : Methanol : water and assayed with the wheat coleoptile test, sections showed the presence of inhibitors all over the paper. This must be due to ether soluble impurities.

In the next experiment two extracts (acid fractions) were prepared from roots of 12 days' old broad beans. One of them had 40 gms. and the other contained 150 gms. of roots. They were chromatographed concurrently and one of the chromatograms (containing 40 gms. of acid root extract) was assayed with oat mesocotyl section test and the resulting histogram is shown in Fig. 17.c. Each of the three growth active zones (accelerator α, IAA and accelerator x) was cut off from the second chromatogram (containing acid fraction of 150 gms. of root) and eluted with 10 mls. of alcohol in 150 ml. conical flask. They were gently shaken on an electric shaker for 5 hours in the dark at room temperature. At the end of this period the alcohol was transferred to the evaporating tube and each flask washed twice

with 5 ml. of alcohol and washings were combined. The alcohol was evaporated off under reduced pressure and residues were taken up in 4 mls. of buffered sucrose solution (generally used for oat mesocotyl assay).

From these stock solutions 1 : 1, 1 : 10, 1 : 100, etc. concentrations were prepared and their activities were tested with oat mesocotyl sections. It would appear from the results (Fig.27) that growth effects of accelerator α, IAA (i.e. substance occurring at IAA zone of chromatogram) and accelerator x take place within a narrow range ofcooncentrations. At higher concentrations they produce inhibitory effects. Arbitrary log. concentrations have been used in Fig.27, each ten times diluted than previous number and the highest is 10^{0} in all three figures.

It is interesting to note that the nature of concentration/growth curve obtained in this experiment for the substance occurring in IAA region, is very different from that obtained with synthetic IAA. The maximumpromotion (about 220% above the controls) that was observed on second dilution is equivalent to about 10 μgm/ml. of synthetic IAA. When this was again diluted (10 times), the growth came down to control level. This disproportionate dilution effect directly indicates that the substance occurring at IAA region of chromatogram may not be identical to synthetic IAA.

Fig.26. Activities of acid growth substances of broad bean roots in different bioassay methods. (Growth responses plotted against Rf. values.)

a. Histogram illustrating pea root assay results of a chromatogram of 50.0 gm. of root tissue from 10 days' old plants.

b. Histogram illustrating wheat coleoptile assay results of a chromatogram of 40.0 gm. of root tissue from 10 days' old plants.

FIG. 26.

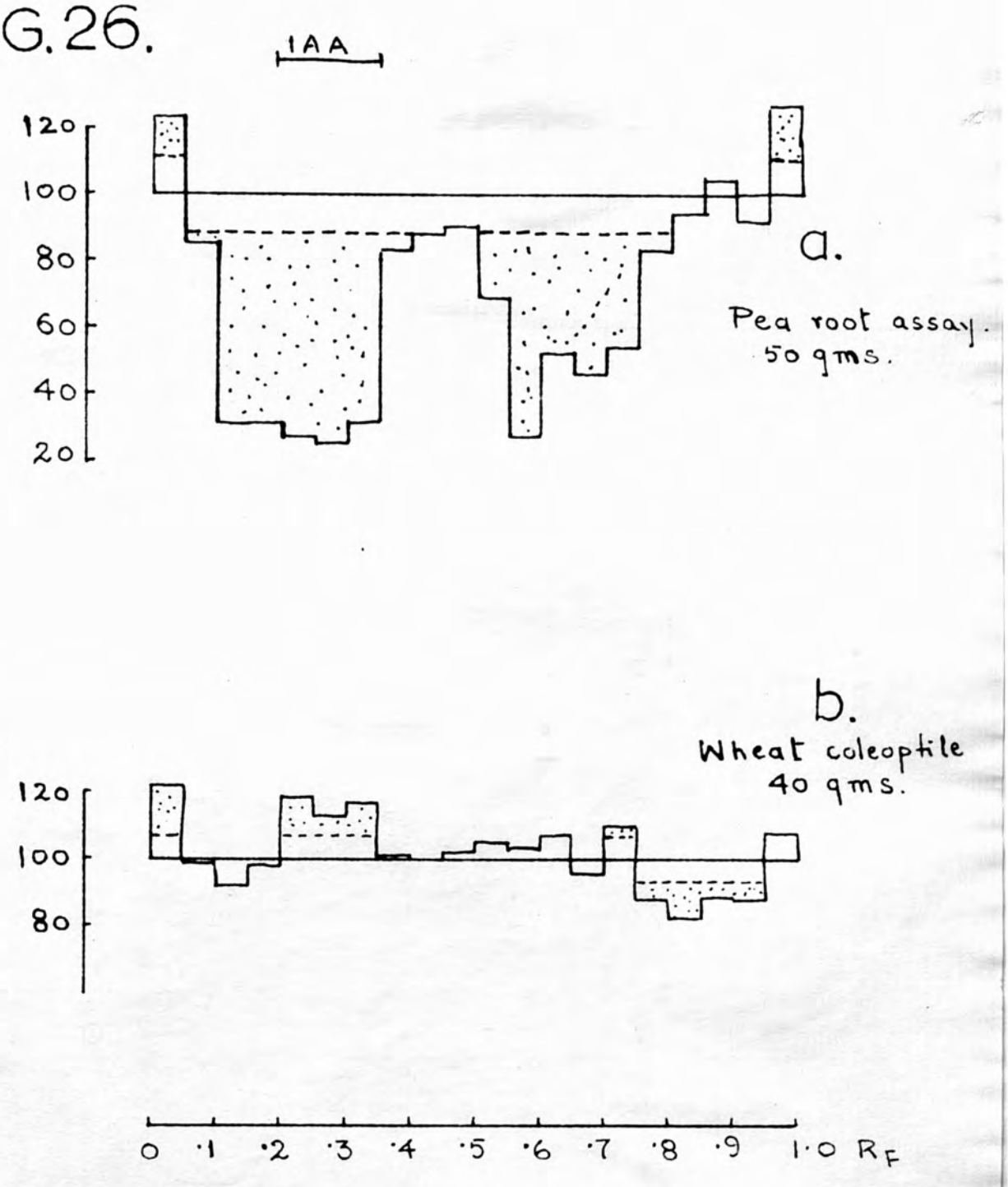
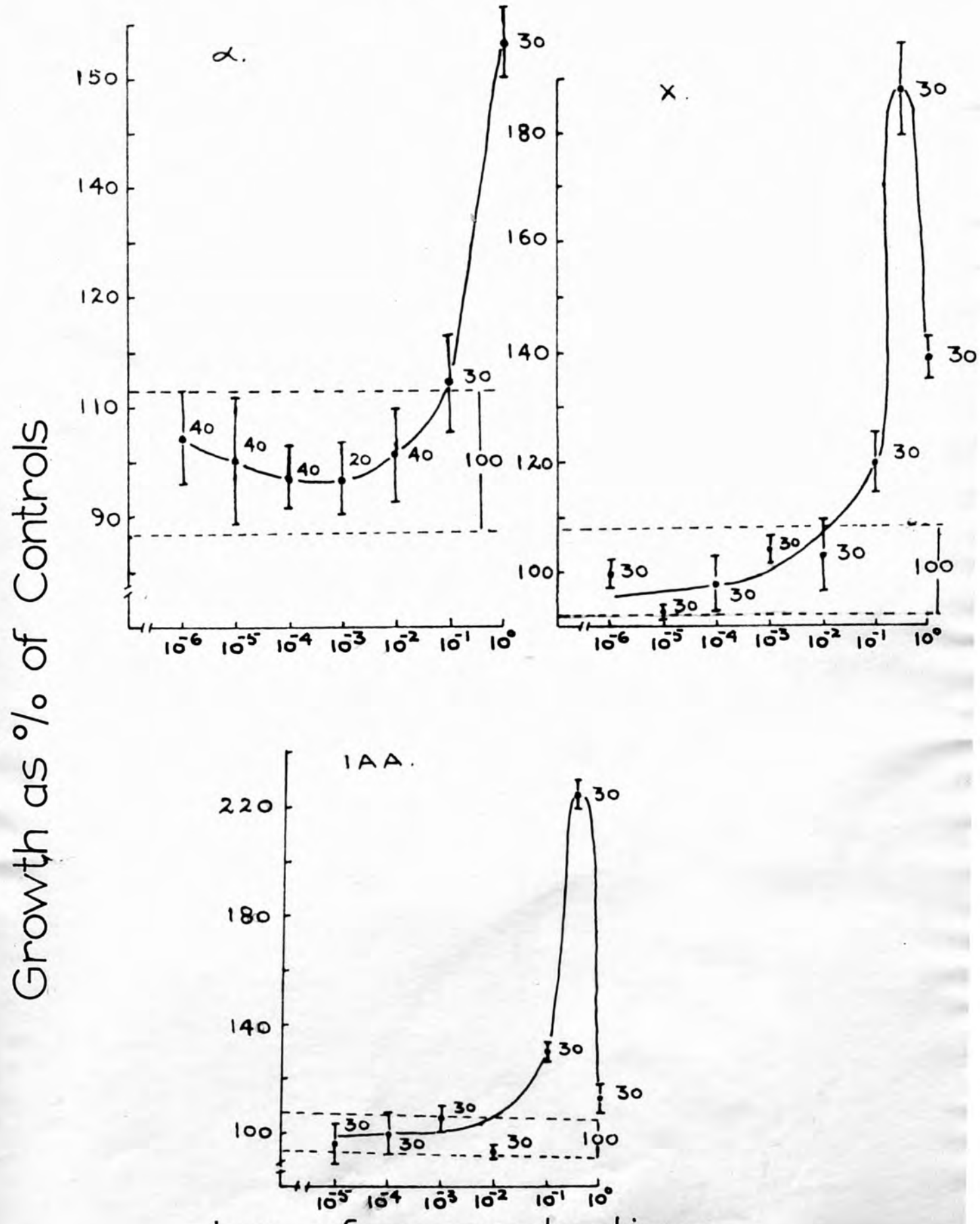

IAA
120
100
80
60
40
20
a.
Pea root assay.
50 qms.
b.
Wheat coleoptile
40 qms.
120
100
80
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 R_F

Fig.27. The activities in oat mesocotyl section extension of accelerator α, IAA (i.e. substance occurring in the IAA zone of chromatograms) and accelerator x obtained from the acid fraction of broad bean root extract. The broken horizontal lines represent 2x standard deviation of control sections (100 sections, 10 sections per growth-vessel). Concentrations of growth substances are presented in arbitrary log. scale as described in the text. Each point represents the mean of a number of determinations, each on 10 sections, the total number of sections being recorded against each mean. The vertical lines through each point extend to ± the standard deviation on either side.

FIG. 27.


α.
150
140
120
110
90
40
40
40
20
40
30
100
30
X.
180
160
140
120
100
30
30
30
30
30
30
100
IAA.
220
180
140
100
30
30
30
30
30
30
100
10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10°
Growth as % of Controls
log. of concentrations

It may be mentioned here that no definite correlation could be observed between U.v. florescence and growth activity on chromatograms. In this particular experiment five florescing zones were observed on the chromatogram with 150 gms. of root extract. They were: (i) Rf. 0.03-.15 (light green); (ii) Rf. 0.17-.3 (deep violet); (iii) Rf. 0.42-0.52 (light violet); (iv) Rf. 0.52-0.66 (light violet); (v) Rf. 0.89-1.0 (light violet).

On another occasion acid fractions of extracts were spotted separately on the same sheet of paper. Two of these contained 40 gms. of root extract from 11 days' old plants and the third was loaded with acid-extract obtained from 220 gms. of roots of the same age. The chromatograms containing 40 gms. of extracts were assayed separately with oat coleoptile and oat mesocotyl assay, and the results are shown in Fig.28 (a and b). In these experiments Victory oats No.2. were used.

It may be seen again that oat coleoptile assay shows similar activity to that observed by Kefford (1955). But in oat mesocotyl assay, no activity due to inhibitor β could be detected and promotion was recorded about the same region for promotor x.

Fig.28. Activities of acid growth substances of broad bean roots in different bioassay methods. (Growth responses plotted against Rf. values.)

a. Histogram illustrating oat coleoptile assay results of a chromatogram of 40 gm. of root tissue obtained from 11 days' old plants.

b. Histogram illustrating oat mesocotyl assay results of a chromatogram containing tissue extract similar to that mentioned in (a).

FIG. 28.

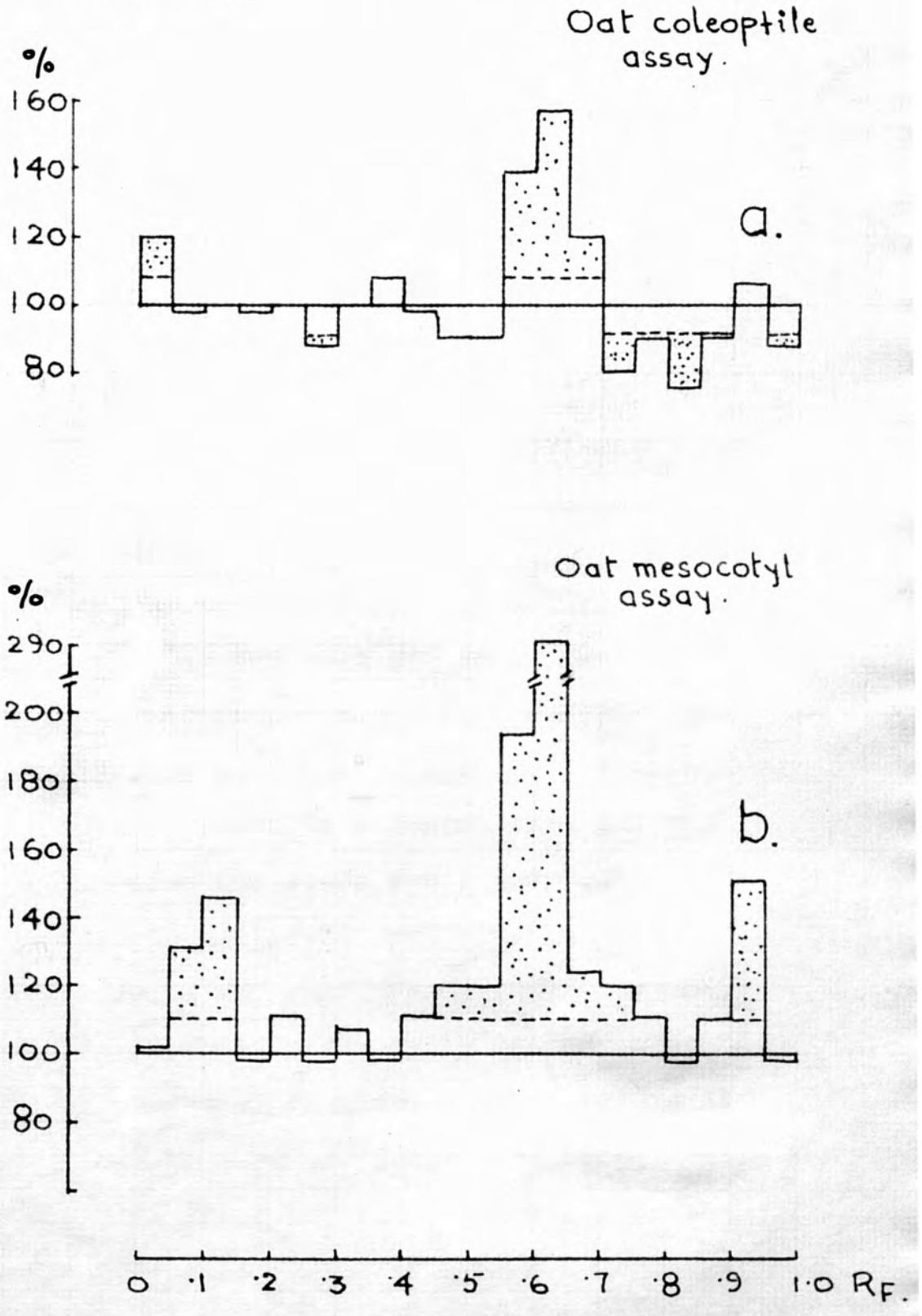
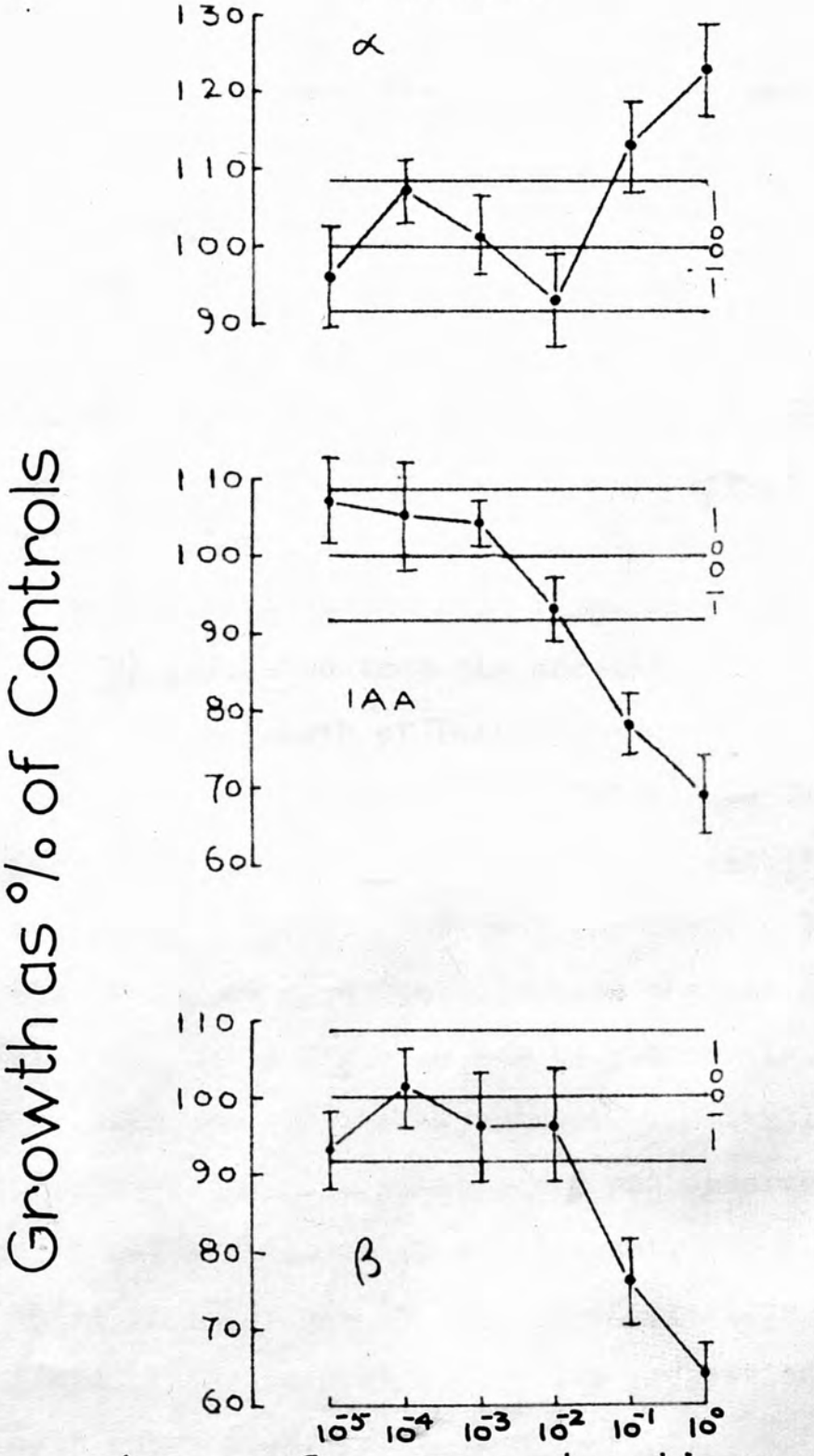

Oat coleoptile assay.
%
160
140
120
100
80
a.
Oat mesocotyl assay.
%
290
200
180
160
140
120
100
80
b.
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F.

Fig.29. The activities in pea root section extension of accelerator α, IAA (i.e. substance occurring in the IAA zone of chromatograms) and inhibitor β, obtained from the acid fraction of broad bean root extract. The horizontal lines above and below 100% level represent 2x standard deviation of control sections (100 sections, 10 sections per growth vessel). Concentrations of growth substances are presented in arbitrary log. scale as described in the text. Each point represents the mean of two determinations, each on 10 sections. The vertical lines through each point extend to $\pm$ the standard deviation on either side.

FIG 29


130
120
110
100
90
α
100
110
100
90
80
70
60
IAA
100
110
100
90
80
70
60
β
100
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0}
Growth as % of Controls
log. of concentrations

The growth active zones as suggested by these two chromatograms were cut off from the third chromatogram containing 220 gms. of acid extract. The following Rf. zones were eluted separately in alcohol, as it was done in the previous experiment: Rf. 0-0.2, Rf. 0.45-0.7, and Rf. 0.75-1.0.

The final residues were taken up in 10 mls. of 1.0 per cent. sucrose solution. These solutions were further diluted as described before. The growth activity of pea root sections was tested in 3 ml. of test solution in 7 cm. Petridishes. The results (Fig.29) indicated that the accelerator α (eluate from Rf. 0-0.2) promotes growth of roots, but the substance occurring at IAA zone (eluate from Rf. 0.45-0.7) and the one immediately after it (eluate from Rf. 0.75-1.0, inhibitor β or substance x) causes inhibition of root sections. Inhibitory action of accelerator α at high concentration was not observed here (Kefford, 1955), which might be due to relatively small quantities of tissues used in the experiment. Slightly higher sensitivity of root sections to inhibitor β was observed, but the difference is not statistically significant.

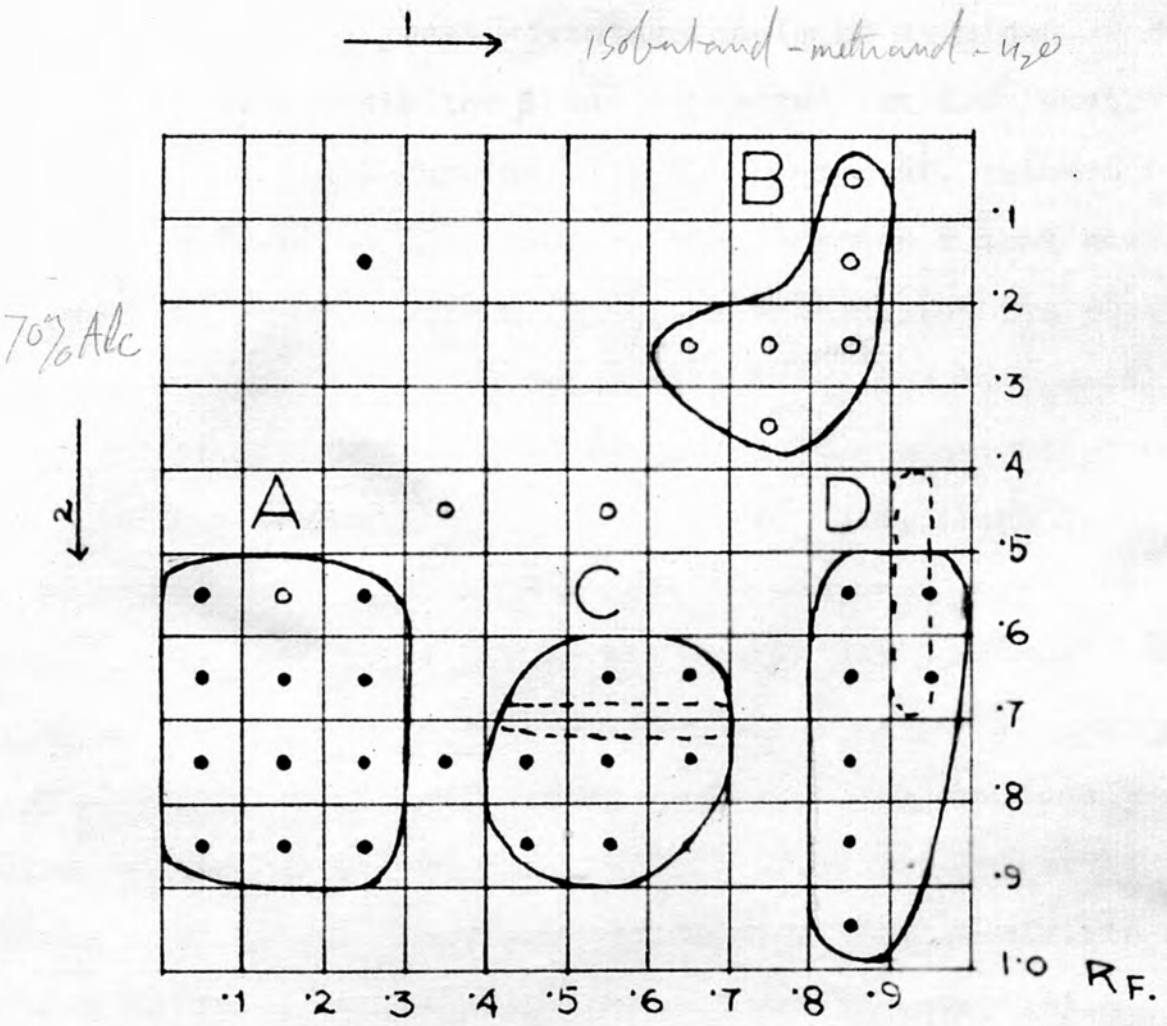
Most of these findings are in support of previous investigations of Kefford (1955) on root elongation properties of the endogenous growth substances.

In the next experiment acid ether extract from 50 gms. of 12 days' old roots was spotted on a two-dimension chromatogram. In the first run Isobutanol : Methanol : water was used as solvent and in the second run 70 per cent. alcohol was used. The paper was divided up into 100 equal parts according to Rf. values and each segment was then assayed with oat coleoptile section test. In Fig.30 significant promotions and inhibitions are plotted and spots are grouped together to represent the position of a growth substance. The experiment was of qualitative nature and no attempt has been made to assess the quantities of the growth substances. Positions of synthetic IAA and IAN as observed from a separate chromatogram undergoing similar chromatographic treatment are presented as dotted lines on the figure.

It may be seen that accelerator α (A in Fig.30) showed rather high Rf. in 70 per cent. alcohol, whereas Rf. value of inhibitor β was relatively lowered (B in Fig.30). Rf. of IAA in 70 per cent. alcohol was found to be 0.7 which nearly corresponds with that observed by Sen and Leopold (Rf. 0.77; 1954), but IAN was found to occur between Rf. 0.4-0.7 whereas the previous authors recorded a Rf. of 0.86 for that substance. However, significant growth promotion was observed exactly at the same Rf. values as that of synthetic IAA (C in Fig.30).

Fig.30. Two-way chromatogram of the acid fraction of broad bean roots. Extract from 50.0 gm. of root tissue from 12 days' old broad bean plants was used in the experiment. The arrows on the figure indicate the directions of the first (in iso-butanol : methanol : water :: 80 : 5 : 15) and the second (in 70% ethanol) runs. Paper was divided up into 100 equal segments and assayed with oat coleoptile sections. Significant promotions and inhibitions (above or below the controls) are shown on the figure. Spots are grouped together and outlined (A, B, C and D) to indicate the positions of growth substances. Areas within dotted lines represent the positions of synthetic IAA and IAN as observed on a separate chromatogram developed in similar solvent systems.

FIG. 30.


1
Isobutanol - methanol - H₂O
70% Alc
2
A
B
C
D
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
RF.
·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9
• accelerator
o inhibitor

It was, however, realised that by using alcoholic solvents in both dimensions no great advantage could be attained. But in this experiment inhibitor β was separated out from another promotor (D in Fig.30) showing slightly higher Rf. value. This promotor could be identical to the promotor x that has been described before. In a single solvent system its presence could not be detected by oat coleoptile assay due to possible masking effect of inhibitor β. Although this promotor occurs at the same position (Rf.) as that of IAN, there is no further proof to show that they are identical substance. Moreover, no positive evidence for the occurrence of IAN was obtained from spraying of chromatograms with Ehrlich's reagent.

The chemical nature of inhibitor β has been critically examined by Housley and Taylor (1958). They have observed that inhibitor of potato tuber peel contains a complex mixture of aliphatic acids. Azelaic acid and a coumarin, scopoletin, were isolated together with a new substance Acid A. Whether or not promotor x is one of these compounds is difficult to predict.

It is tempting to believe that inhibitor β of root tissue also constitutes similar substances. The results presented here indicate the presence of a promotor (of oat coleoptiles) almost at the same Rf. values as that of an inhibitor. But

whether accelerator x of oat mesocotyl sections is identical to inhibitor β of pea root and oat coleoptile sections, or the other promotor D (of Fig.30), cannot be established on the basis of the data presented here.

(c) Distribution of acid growth substances in broad bean roots at different ages of the seedlings

In this section, chromatographic analysis of acid growth substances from 0.5 cm. root tips, the rest of the tap roots and lateral roots was carried out. The investigation was extended towards evaluation of the auxin picture in these three parts of the root at different ages of the seedling.

The results of these investigations have been illustrated as histograms in Figs. 31-39.

It is noticeable in these histograms that accelerator α, the substance occurring at the IAA region of chromatograms and accelerator x are the three main substances which are changing in concentrations with age. In a few cases stimulations or inhibitions were recorded in the Rf. values not corresponding to these substances and their activities are noted in the Table 8 (Appendix). Oat mesocotyl assay has been extensively used in this investigation, but pea root assay was also used occasionally. However, when pea root assay was used, promotion due to accelerator x could not be detected, but inhibition due

to inhibitor β was prominent. Inhibition that was noted in Fig.31.b. near the IAA zone could be due to inhibitory concentration of that substance.

Activity (in μgm/hgm. IAA eq.) of each substance and the actual positions of growth active zones on chromatograms are shown under each experiment in Table 8 (Appendix). But graphical presentation of activity of each substance against the age of the seedlings was more revealing. In Fig.40.a., IAA (i.e. substance occurring at IAA region of chromatograms) concentration in 0.5 cm. root tips has been plotted against time. It could be seen that in young roots (3-4 days) tips high concentration of the substance was noted and it gradually fell as the age increased. It is interesting to note that at a certain stage no IAA (34 days, Fig.38.a.) could be found.

In the root stump, however, the picture (Fig.4.b.) was somewhat different. In young roots (4 days) relatively low concentration of IAA (0.023 μgm/hgm.) was observed which gradually increased to a maximum (0.527 μgm/hgm IAA eq., 0.46 μgm/hgm IAA eq.) in 18-20 days and then came down to low values (0.054 μgm/hgm IAA eq., 0.079 μgm/hgm IAA eq.) on the 25th and 34th day. But in no case IAA concentration got anywhere near those in the tips. (Fig.41)

IAA in lateral roots (Fig.41) shows a similar picture to what was observed in the tap root tips. In 10 days' old roots 0.04 and 0.46 µgm/hgm. IAA eq. of this substance was recorded in two separate experiments and this gradually fell with time to a minimum of 0.0016 and 0.0025 µgm/hgm. IAA eq. on the 34th day. The IAA in lateral roots was smaller than in the stumps in all cases.

It could be observed again that, in general, in tap root tips concentration of IAA was of highest order, next highest was in root stumps and in the lateral roots the lowest level was recorded.

Accelerator α, in the tap root tips (Fig.42.a.) also increased with age up to the 18th day and fell to a zero value on the 25th and 34th day. But the same substance in stumps maintained a steady low value (around 0.02 µgm/hgm.) up to the 18th day and fell to zero on the 25th day (Fig.37.b.). No trace of this substance could again be observed on the 34th day (Fig.38.b.).

Accelerator α, in the whole of laterals (Fig.42.b.) also maintained a very low (0.002 µgm/hgm. IAA eq.) steady concentration in all ages of roots.

Accelerator x, however, presents a very interesting picture. In the young as well as in old tap root tips very high

concentration (around 10 µgm/hgm IAA eq.) of this substance was noted. On many occasions the concentration of the substance was too high to estimate and consequently graphical presentation of results was not possible. In the root tips of old plants where no trace of accelerator α or IAA could be found, this substance was present in large amounts. Often its concentration was too high and went above the limits of standard calibration curve. In the stumps, on the other hand, relatively low concentration was observed. Here (Fig.42.c.) the concentration increased with age up to 18 days and came down to low values thereafter. Amounts of accelerator x in the laterals was more or less constant over different ages and a steady concentration around 0.03 µgm/hgm. IAA eq. was maintained.

On the basis of these findings it would appear that auxin growth relationships in different parts of the root are by no means very simple. The basic idea that in older roots supra-optimal concentration auxin (presumably IAA) is maintained does not get any support from the present findings. The main bulk of growth substance in old root tips is accelerator x (of mesocotyl sections), the role of which in root growth is still uncertain. But a logical deduction will be that in old roots

Fig.31. Oat mesocotyl assay of chromatograms of acid fractions of root tips (0.5 cm. from the apex) and root stumps (rest of the root excluding the tip), obtained from 4 days' old broad bean plants.

A. 3.0 gm. of root tip material used for extraction.

B. 31.0 gm. of root stump material used for extraction.

(Growth responses plotted against Rf. values.)

FIG. 31.

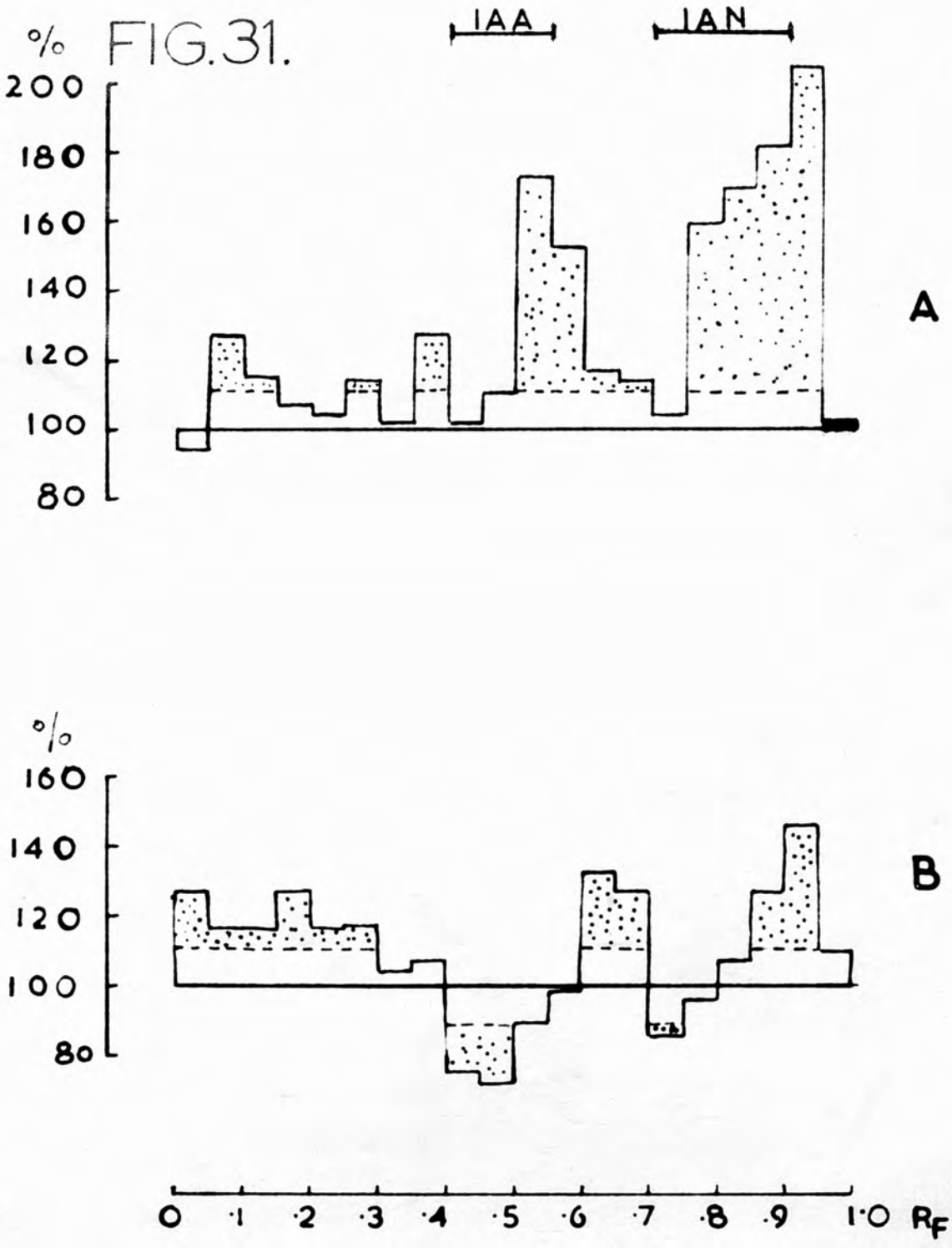

%
IAA
IAN
200
180
160
140
120
100
80
A
4 Days.
%
160
140
120
100
80
B
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
RF

Fig.32. Pea root assay of chromatogram of acid fraction of root tips (0.5 cm. from the apex) obtained from 4 days' old broad bean plants. (Growth responses plotted against Rf. values.)

FIG. 32.

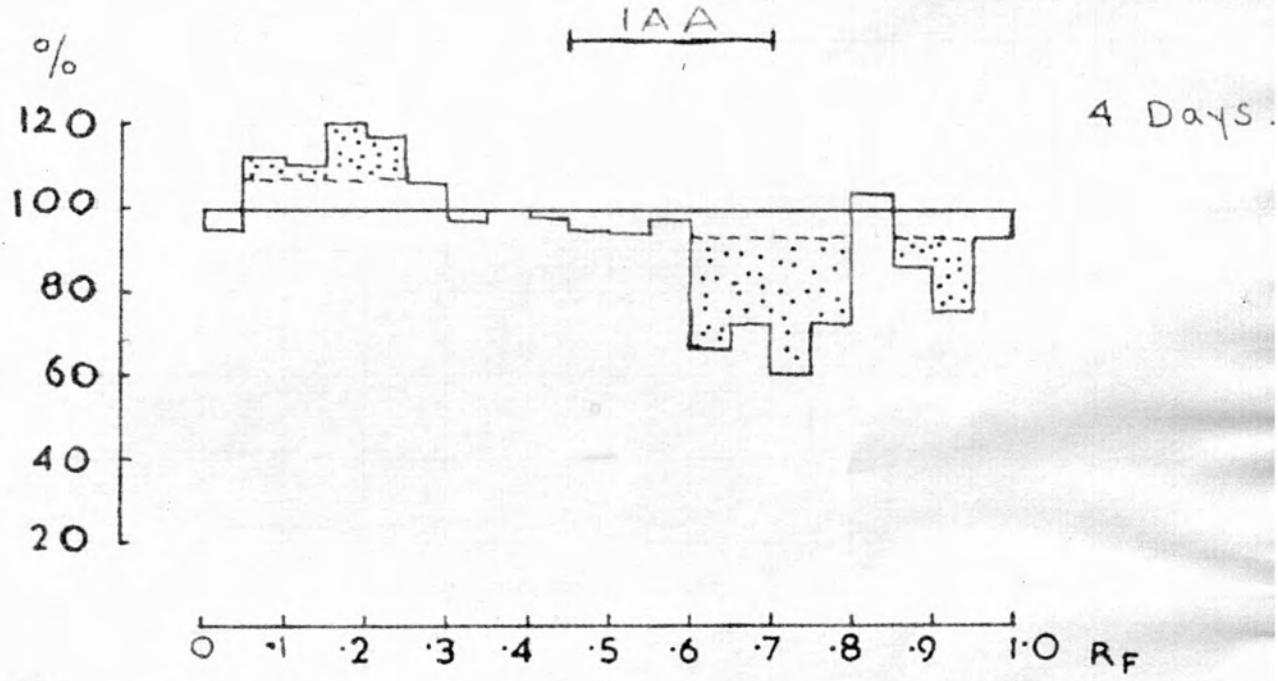


Fig.33. Oat mesocotyl assay of chromatograms of acid fractions of root tips (0.5 cm. from the apex), root stumps and root laterals obtained from 10 days' old broad bean plants. (Growth responses plotted against Rf. values.)

A. 1.5 gm. of root tip material used for extraction.

B. 35.0 gm. of root stump material used for extraction.

C. 32.0 gm. of root laterals used for extraction.

FIG. 33.

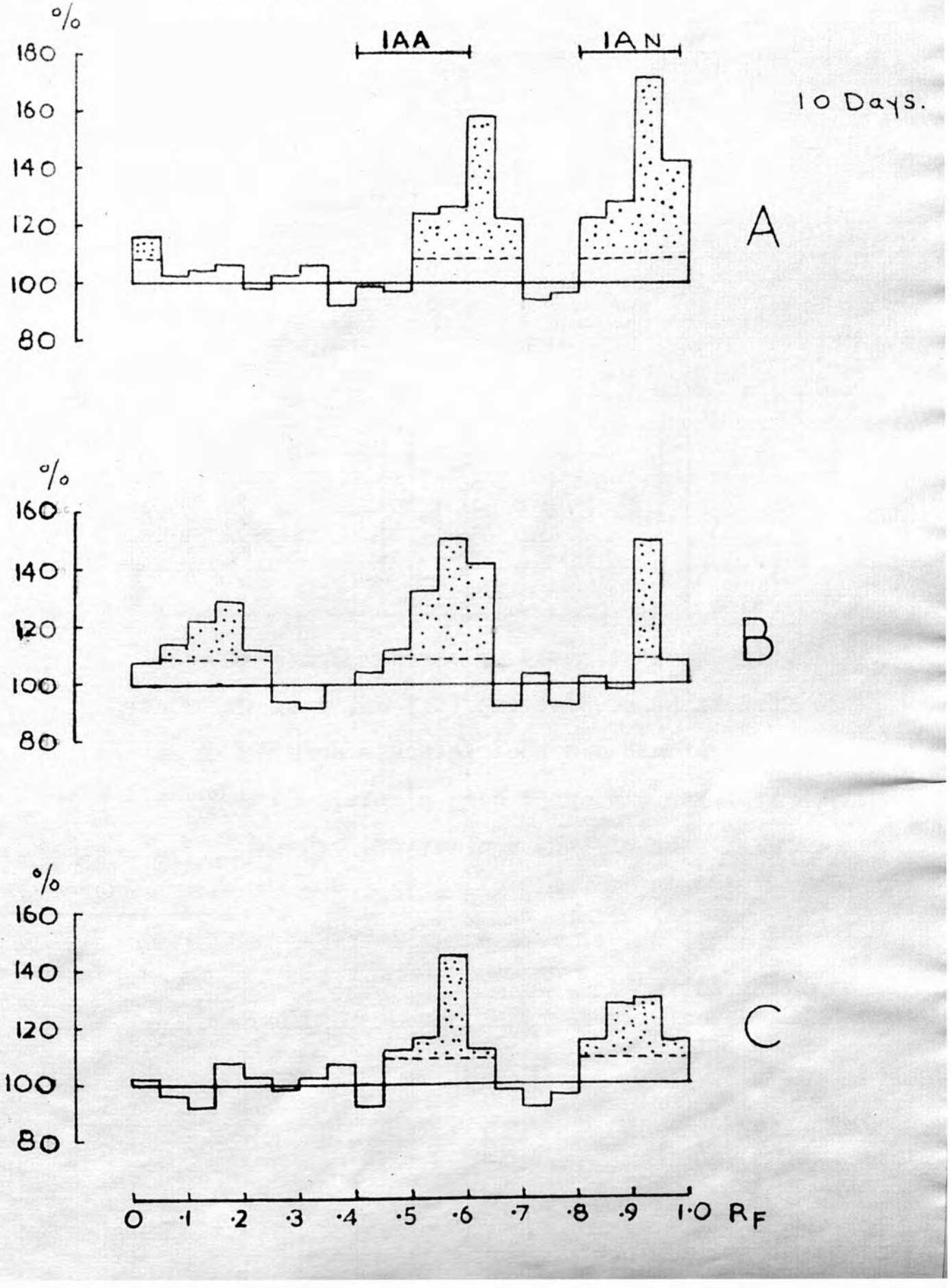

%
180
160
140
120
100
80
IAA
IAN
10 Days.
A
%
160
140
120
100
80
B
%
160
140
120
100
80
C
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
RF

Fig.34. Oat mesocotyl assay of chromatograms of acid fractions of root tip (0.5 cm. from the apex), root stumps and root laterals obtained from 15 days' old broad bean plants. (Growth responses plotted against Rf. values.)

A. 1.5 gm. of root tip material used for extraction.

B. 30.0 gm. of root stump material used for extraction.

C. 30.0 gm. of root laterals used for extraction.

FIG 34

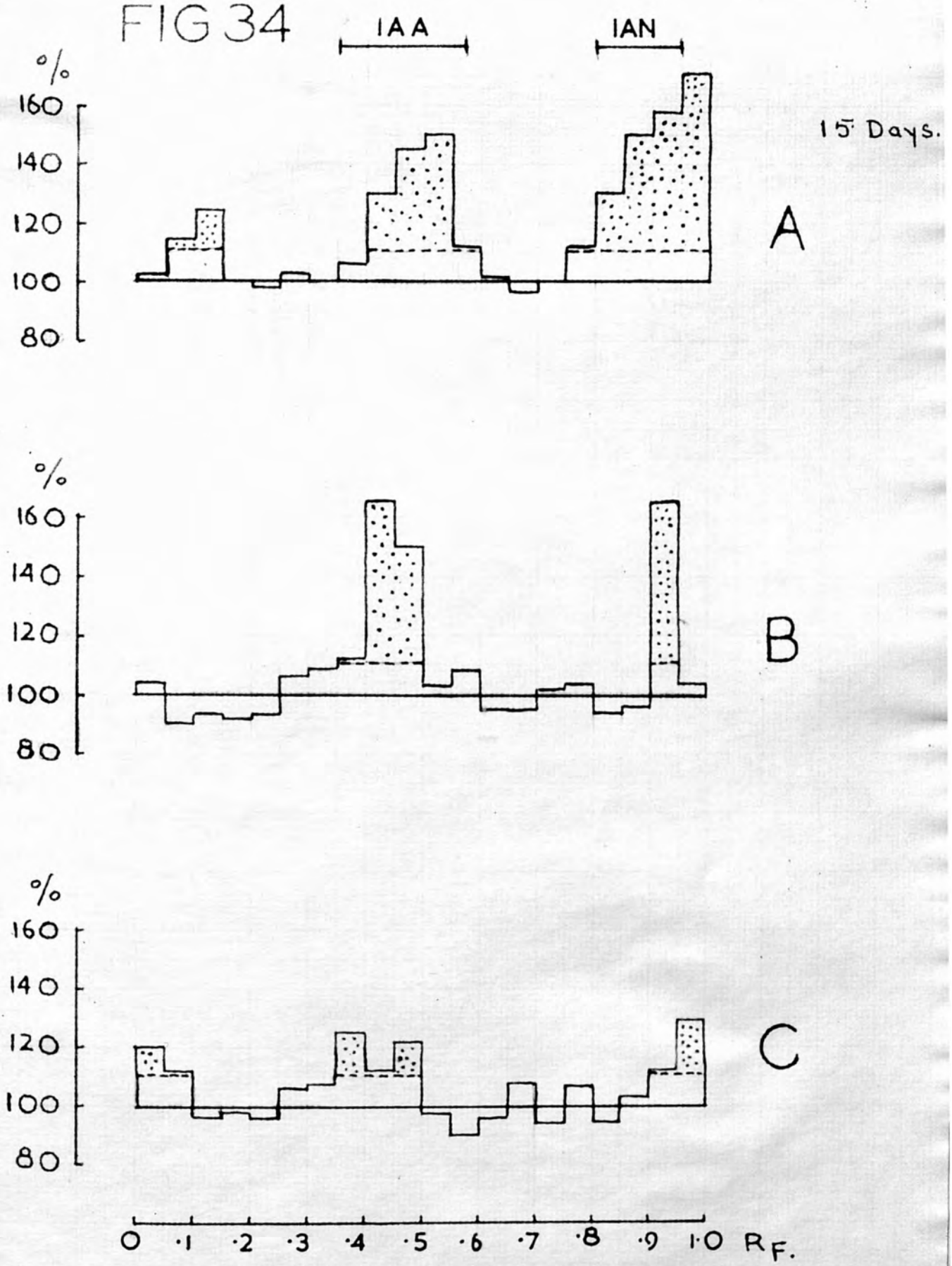

IAA
IAN
%
160
140
120
100
80
A
15 Days.
%
160
140
120
100
80
B
%
160
140
120
100
80
C
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.35. Oat mesocotyl assay of chromatograms of acid fractions of root tips (0.5 cm. from the apex), root stumps and root laterals obtained from 16 days' old plants. (Growth responses plotted against Rf. values.)

A. 1.1 gm. of root tip material used for extraction.

B. 35.0 gm. of root stump material used for extraction.

C. 35.0 gm. of root laterals used for extraction.

FIG 35

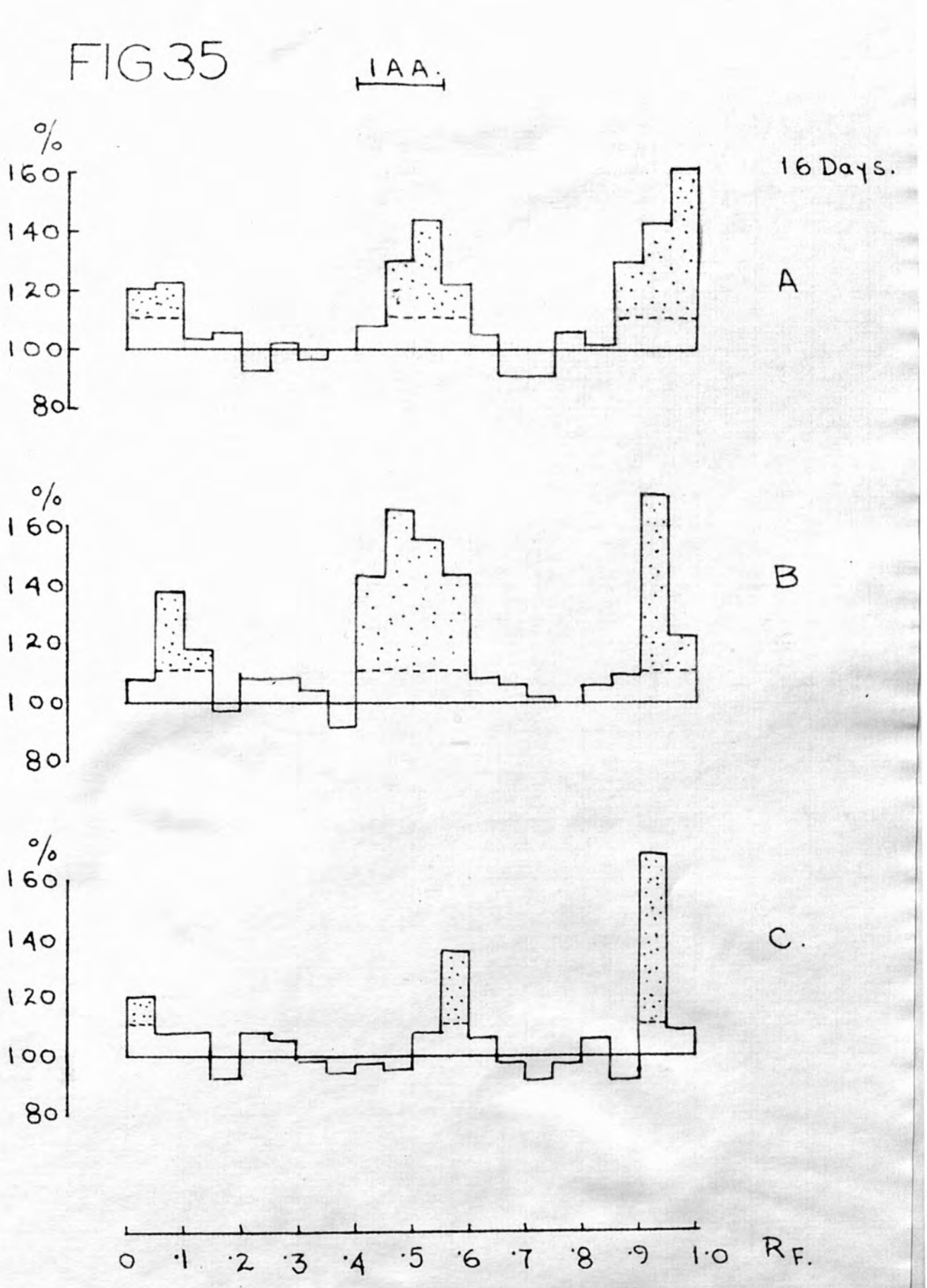

IAA.
%
160
140
120
100
80
16 Days.
A
%
160
140
120
100
80
B
%
160
140
120
100
80
C.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.36. Oat mesocotyl assay of chromatograms of acid fractions of root tips (0.5 cm. from the apex), root stumps and root laterals obtained from 18 days' old broad bean plants. (Growth responses plotted against Rf. values.)

A. 1.5 gm. of root tip material used for extraction.

B. 31.0 gm. of root stump material used for extraction.

C. 35.0 gm. of root laterals used for extraction.

FIG 36

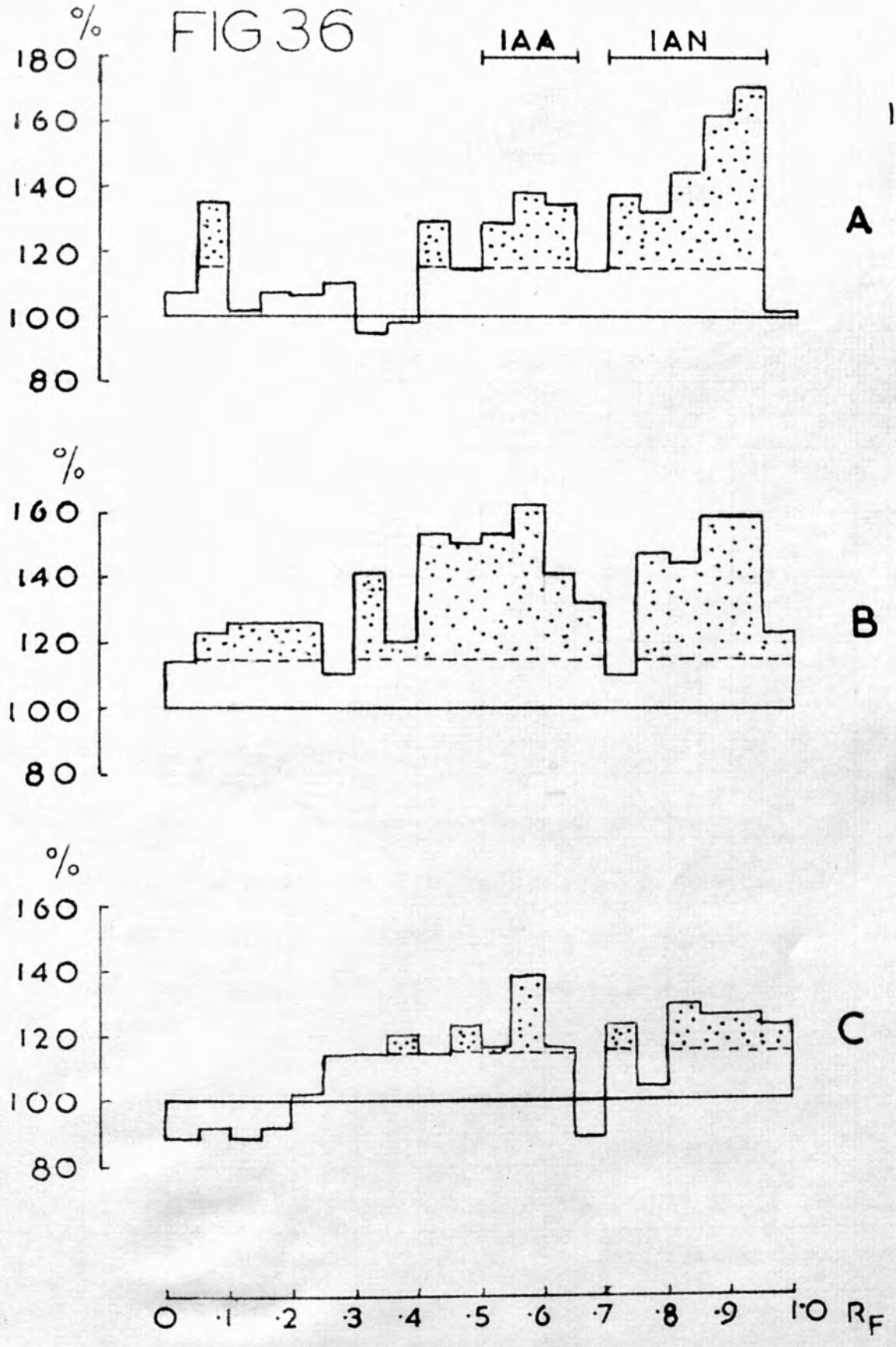

%
180
160
140
120
100
80
IAA
IAN
18 Days.
A
%
160
140
120
100
80
B
%
160
140
120
100
80
C
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 R_F

Fig.37. Oat mesocotyl assay of chromatograms of acid fractions of root tips (0.5 cm. from the apex), root stumps and root laterals obtained from 25 days' old broad bean plants. (Growth responses plotted against Rf. values.)

A. 1.5 gm. of root tip material used for extraction.

B. 35.0 gm. of root stump material used for extraction.

C. 35.0 gm. of root laterals used for extraction.

FIG 37

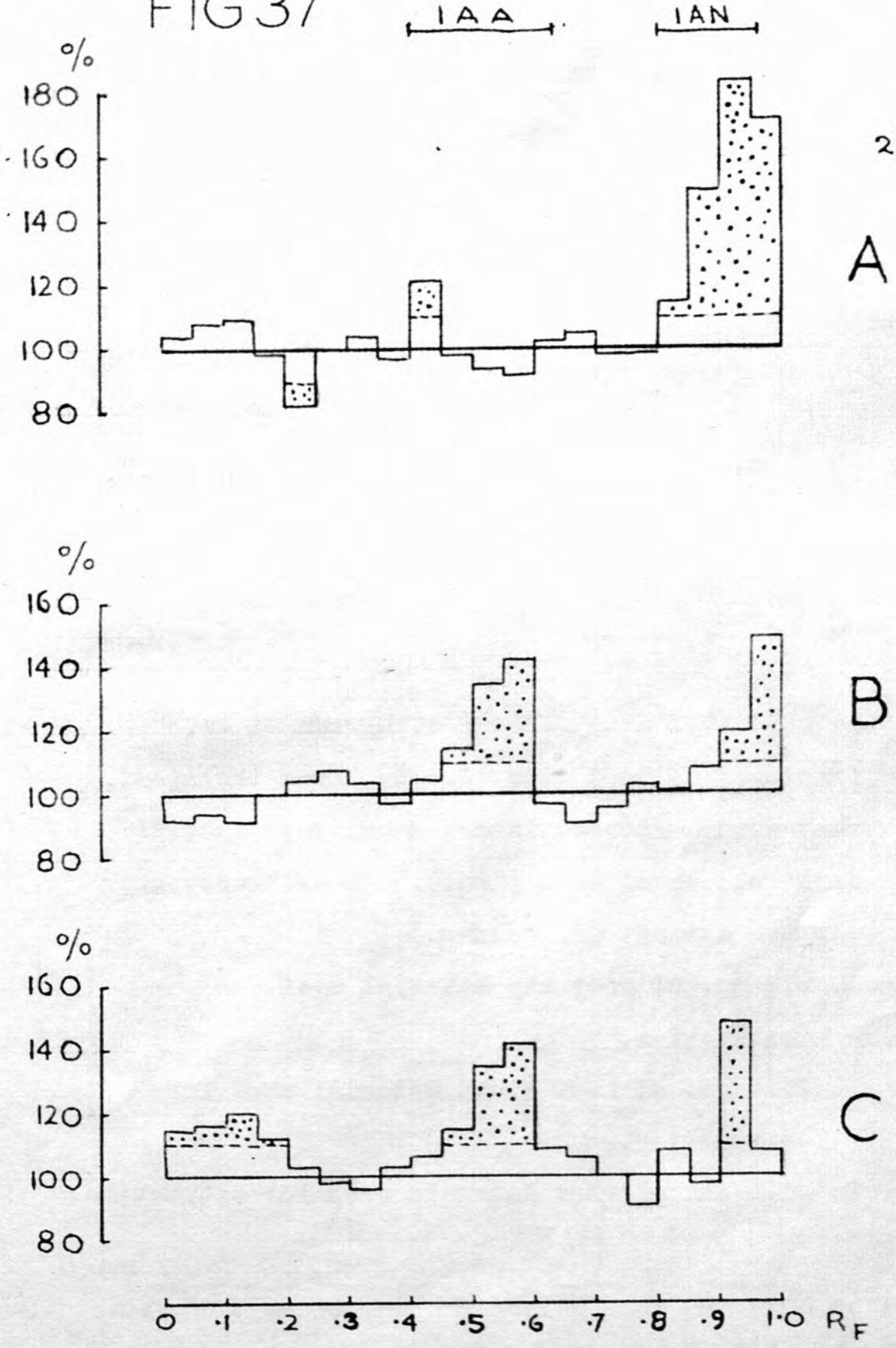

IAA
IAN
%
180
160
140
120
100
80
25 Days.
A
%
160
140
120
100
80
B
%
160
140
120
100
80
C
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Fig.38. Oat mesocotyl assay of chromatograms of acid fractions of root tips (0.5 cm. from the apex), root stump and root laterals obtained from 34 days' old broad bean plants. (Growth responses plotted against Rf. values.)

A. 0.7 gm. of root tip material used for extraction.

B. 20.0 gm. of root stump material used for extraction.

C. 31.0 gm. of root laterals used for extraction.

FIG. 38.

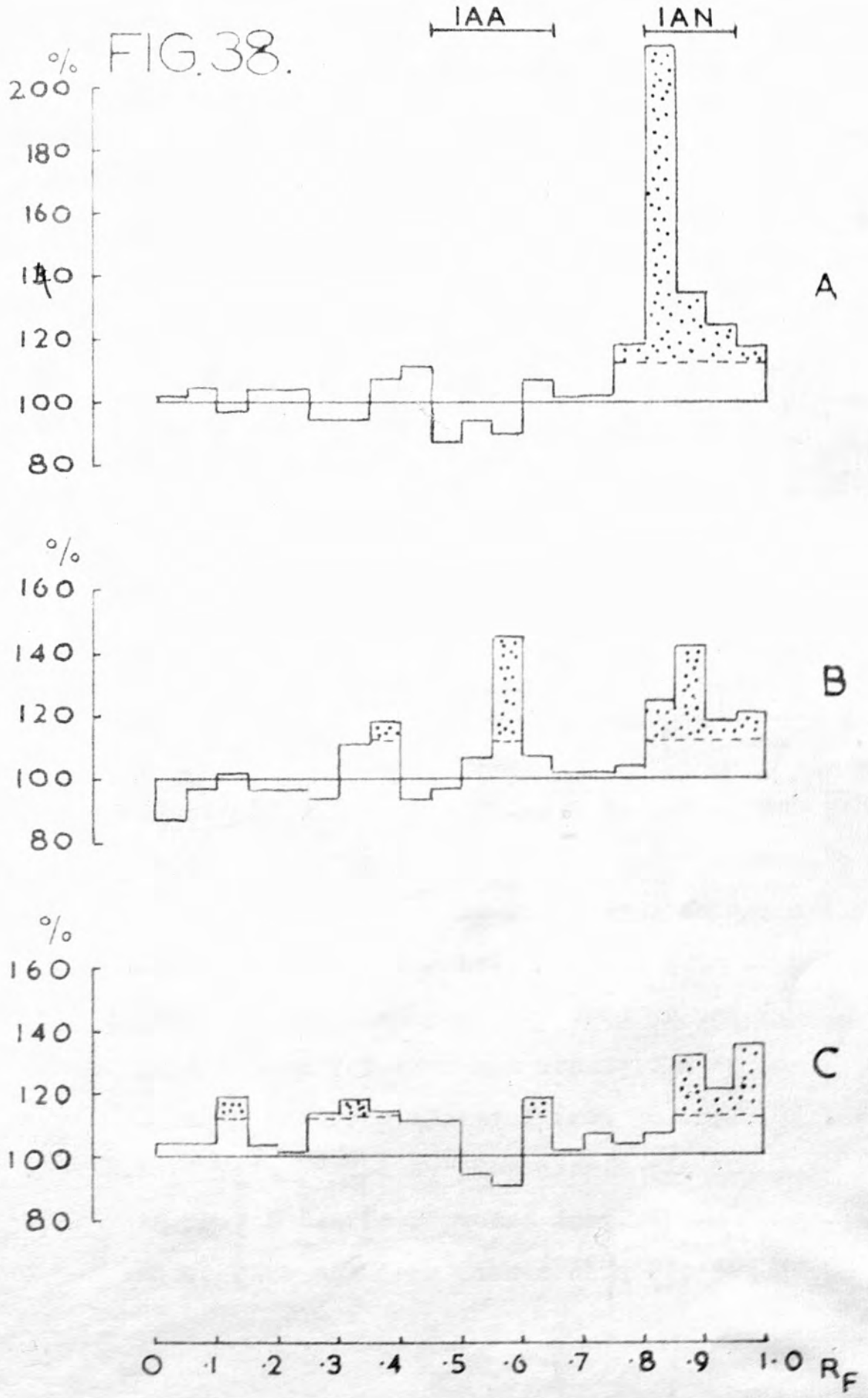

IAA
IAN
%
200
180
160
140
120
100
80
A
34 Days.
%
160
140
120
100
80
B
%
160
140
120
100
80
C
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 R_F

Fig.39. Pea root assay of chromatograms of acid fractions of root tip (0.5 cm. from the apex) and root laterals obtained from broad bean plants. (Growth responses plotted against Rf. values.)

A. 1.6 gm. of root tip material obtained from 3 days' old plants was used for extraction.

B. 30.0 gm. of root laterals obtained from 18 days' old plants were used for extraction.

C. 35.0 gm. of root laterals obtained from 34 days' old plants were used for extraction.

FIG.39.

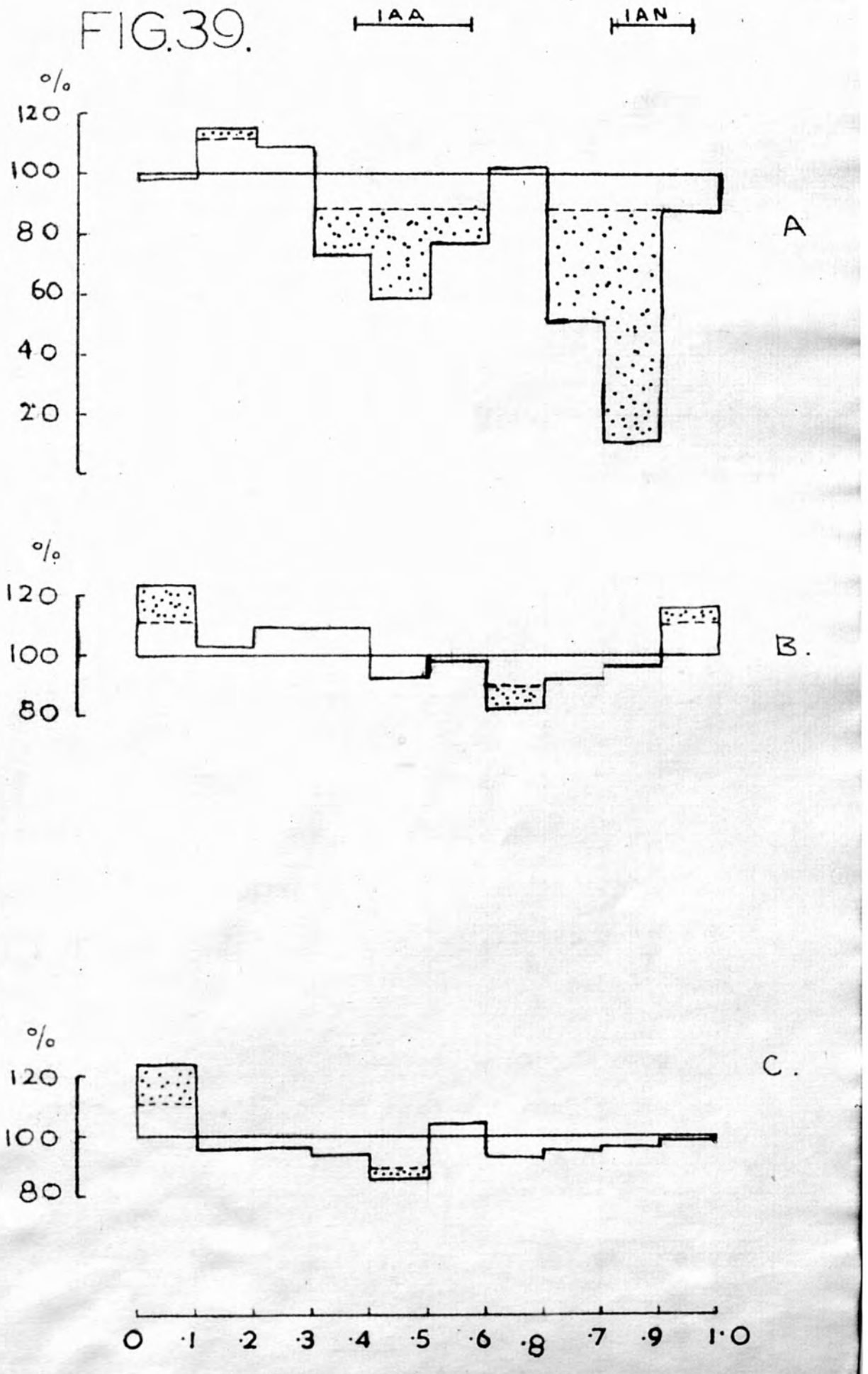

IAA
IAN
%
120
100
80
60
40
20
A
%
120
100
80
B.
%
120
100
80
C.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·8 ·7 ·9 1·0

Fig.40. a. Quantitative changes in the amounts of IAA (i.e. substance occurring at the IAA region of chromatograms) in tap root tips (0.5 cm. from the apex) at different ages of broad bean plants.

b. Quantitative changes in the amounts of IAA in the root stumps, at different ages of broad bean plants.

In both figures, Abscissae: age of seedlings in days, starting from the date of sowing. Ordinates: amounts of IAA in μgm/hgm IAA equivalents.

FIG. 40.

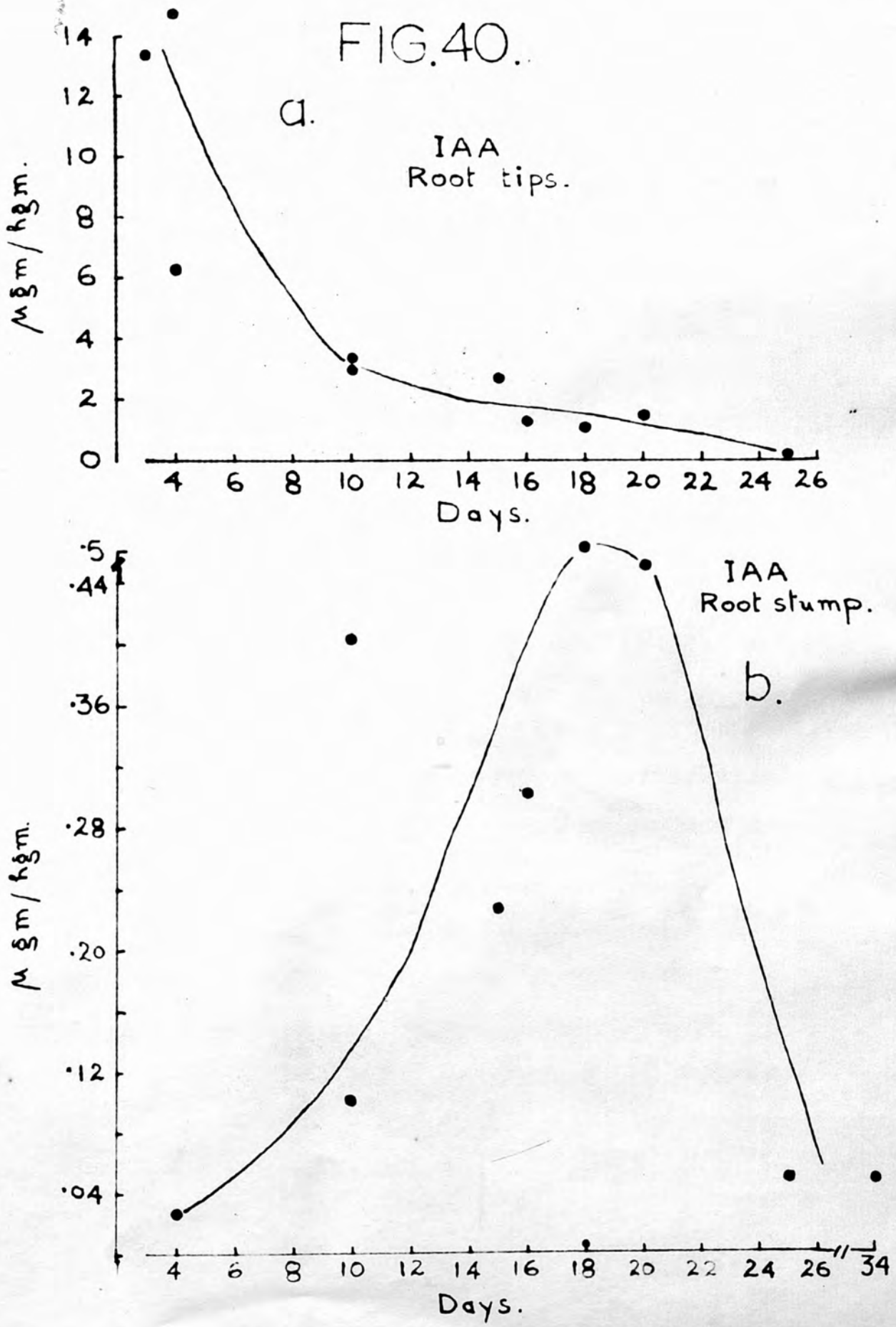

a.
IAA
Root tips.
mgm/kgm.
14
12
10
8
6
4
2
0
4 6 8 10 12 14 16 18 20 22 24 26
Days.
b.
IAA
Root stump.
mgm/kgm.
·5
·44
·36
·28
·20
·12
·04
4 6 8 10 12 14 16 18 20 22 24 26 34
Days.

Fig.41. Quantitative changes in the amounts of IAA in the lateral roots (whole of lateral) at different ages of broad bean seedlings. The arrow on the figure indicates the course of the curve. Abscissa: age of seedlings in days starting from the date of sowing. Ordinate: amount of IAA in μgm/hgm IAA equivalent.

FIG. 41.

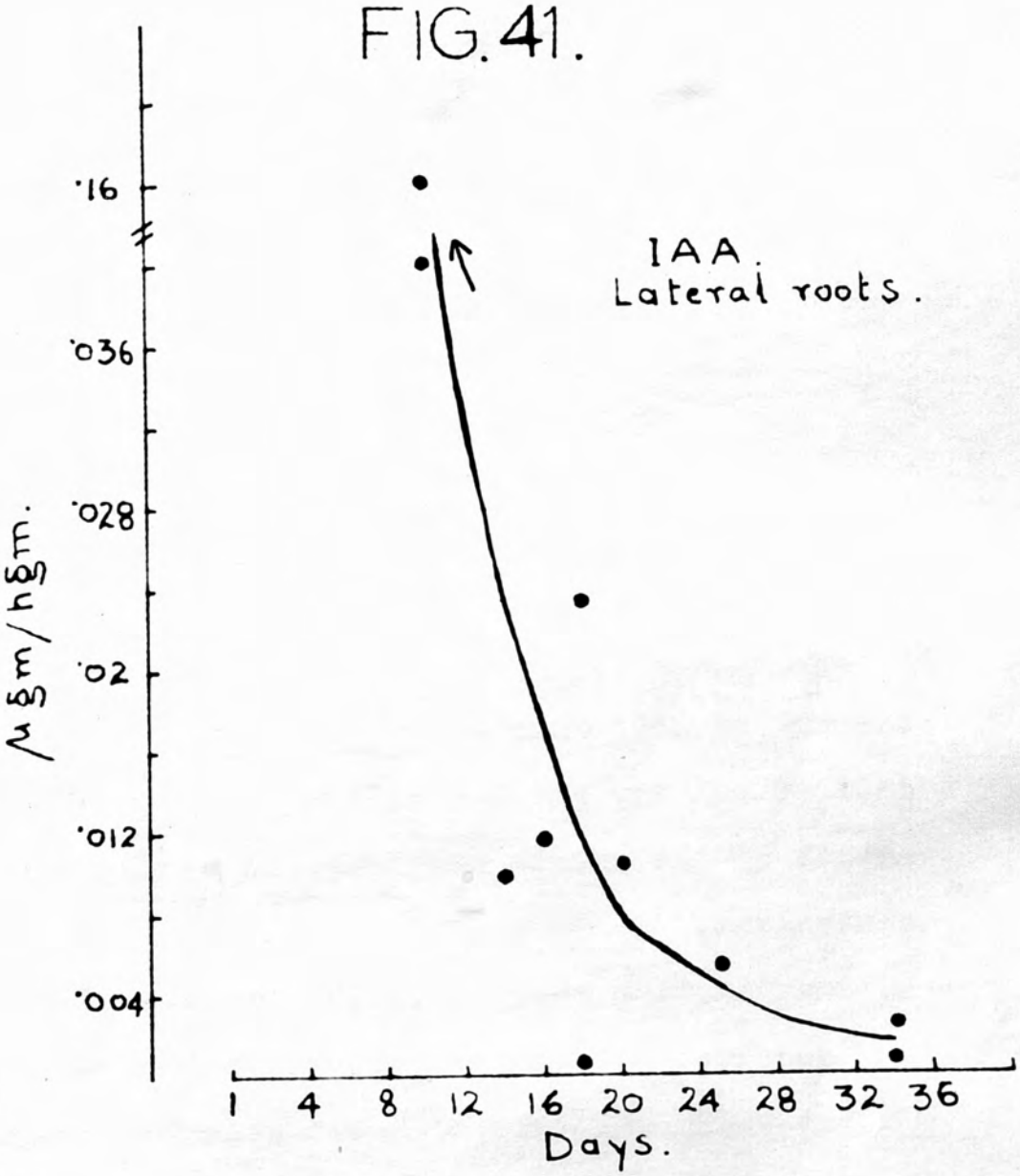

·16
·036
·028
·02
·012
·004
µgm/hgm.
IAA.
Lateral roots.
1 4 8 12 16 20 24 28 32 36
Days.

Fig.42. In each figure (a, b and c) the abscissa represents the age of seedlings in days, starting from the date of sowing, and the ordinate represents the amount of the growth substance in µgm/hgm IAA equivalent.

a. Quantitative changes in the amounts of accelerator α in the root tips and root stumps at different ages of broad bean seedlings.

b. Quantitative changes of accelerator in the lateral roots at different ages of broad bean seedlings.

c. Quantitative changes of accelerator × in the lateral roots at different ages of broad bean seedlings.

FIG. 42.

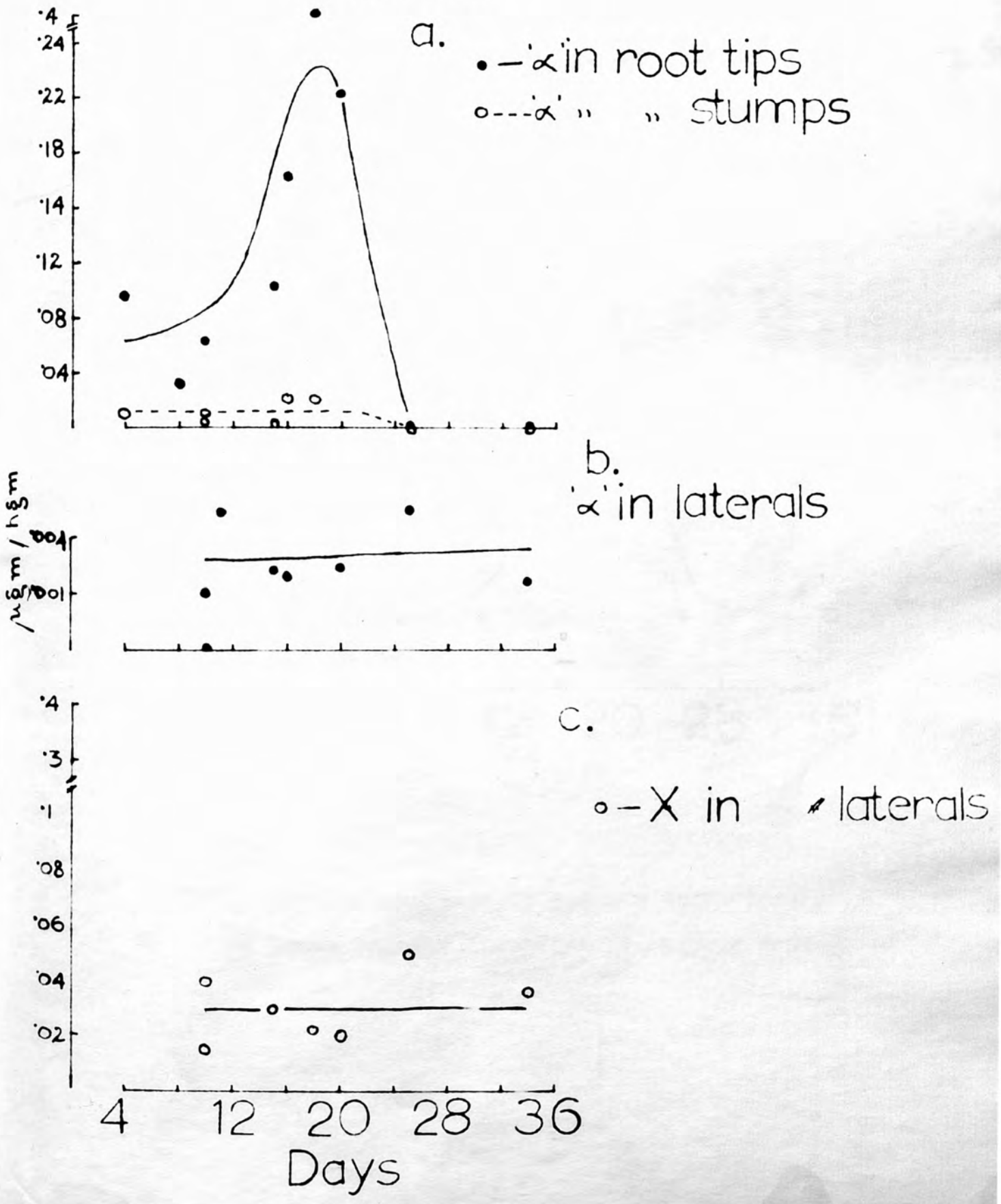
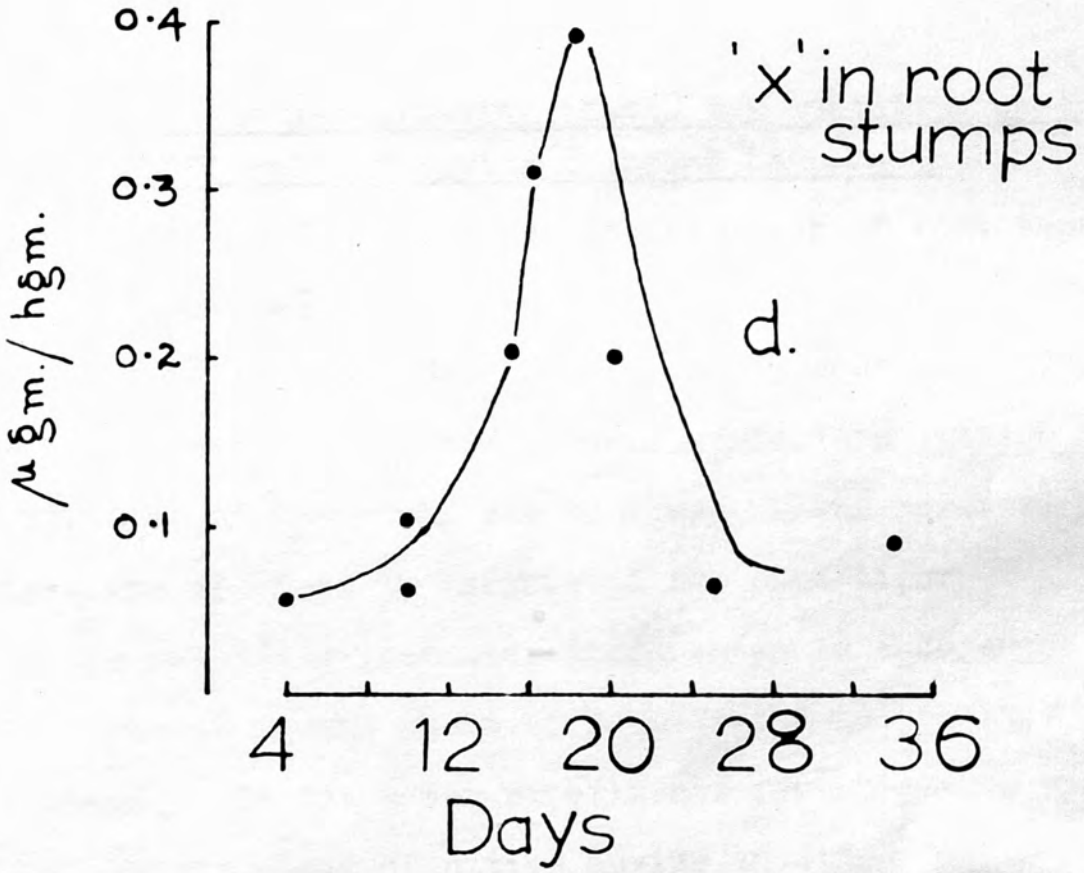

a.
•—'α' in root tips
o---'α' " " stumps
·4
·24
·22
·18
·14
·12
·08
·04
b.
'α' in laterals
µgm / µgm
·004
·001
c.
o—X in laterals
·4
·3
·1
·08
·06
·04
·02
4
12
20
28
36
Days

Fig.42. (contd.)

d. Quantitative changes of accelerator x in the root stumps, at different ages of broad bean plants.

FIG 42(contd.)



growth-effects of external application of any compound will be determined by possible antagonism or synargism with the promotor x in the elongating cells of the tap root tip. However, in the section devoted to discussions, attempts will be made to explore further possibilities.

(d) Effects of the decapitation of the tap root tips on the endogenous levels of auxins in broad bean roots

The implications related to decapitation of root tips has already been pointed out in the introductory chapter. The effects of decapitation are also intimately connected with the growth and formation of lateral roots. In this section the main objective of the study was to find out the hormonal readjustments of roots in absence of tap root tips.

In the initial experiments differences in endogenous levels of auxins in the whole of decapitated and control roots were studied. In the later experiments investigation was extended towards assay of native auxins in different parts of control and decapitated roots. The quantities of growth substance that were observed in different experiments of this series are presented in Table 9 (Appendix).

Plants were grown for these experiments on sand for 3 days and then they were transferred to aerated water tanks. The root tips (0.5 cm.) of one set of plants were excised with a

sharp razor-blade at this stage and were allowed to grow in water-tanks. The control plants (with root tips) were also grown under identical conditions in separate tanks.

The roots were harvested on the 9th day and the acid fractions of extracts of both control and treated plants (without tap root tips) were chromatographically analysed. Neutral fractions were also analysed in one experiment. In Fig.43 the assay (oat mesocotyl assay) results of 30 gm. of control and decapitated root tissue are presented as histograms. In the acid fraction of control roots, 0.83 µgm/hgm. IAA eq. of accelerator α, 0.43 µgm/hgm. IAA eq. of IAA and 0.4 µgm/hgm. IAA eq. of accelerator x was recorded. In the decapitated roots, 0.001 µgm/ghm. IAA eq. of accelerator α, 0.044 µgm/hgm. IAA eq. of an additional promotor (perhaps part of α), 1.51 µgm/hgm. IAA eq. of IAA and 1.88 µgm/hgm. IAA eq. of promotor x was recorded. The total acid auxin in control roots was 0.91 µgm/hgm. IAA eq. in control roots and 3.435 µgm/hgm. IAA eq. in decapitated roots. In the neutral fraction of control extracts no growth activity was observed and in the decapitated neutral extract slight promotion just above the significance limit was observed at Rf. 0-.1.

It was observed from this experiment that decapitated roots contain more auxins than control roots when they are allowed to grow for some time. This experiment was repeated again using pea root section test. The histograms of acid fractions of

control and decapitated plants are shown in Fig.44 a. and b. respectively. It was again observed that in 9 days' old decapitated roots contain more auxin than the control. (Results of assay of chromatograms are present in Table 12, Appendix.)

More interesting results were obtained in the next experiment. Here the following parts of 9 days' old control and decapitated roots were analysed:

a. lateral root tips of control plants.

b. lateral root tips of decapitated plants.

c. root stumps of control plants with tap root tips.

d. root stumps of decapitated plants.

The quantitative results have been presented in Table 9 (Appendix, Expt. 70). The immediate effect of decapitation was manifested in the auxin picture of lateral tips. It was observed that individual auxins, as well as total acid auxin in the later tips was increased in the decapitated roots. IAA was 0.26 µgm/hgm IAA eq. in the control tips (of lateral roots) but 2.5 µgm/hgm IAA eq. was noted in decapitated ones. Similarly, total acid auxin in control tips (of lateral roots) was 0.42 µgm/hgm IAA eq., whereas in decapitated tips it shot up to 3.33 µgm/hgm IAA eq.

In the stump, the effects were rather obscure. In the control plants of this experiment the tap root tips were also

included and therefore could not be strictly compared to the root stump of decapitated roots. Nevertheless, more IAA and promotor x was observed in the decapitated stumps. The movement of the control histogram was rather irregular on this occasion. Slight inhibition was observed at low Rf. values and an additional zone of stimulation was noted at Rf. 0.75-0.9. (Table 9, Appendix.)

In order to substantiate these observations the experiment was repeated again. (Expt. 70.A; Table 9, Appendix.) Here the control and decapitated plants were grown for 16 days. In the treated plants, decapitation was done on the 5th day of sowing. The tap root tips of control plants were separated from stumps and assayed separately.

The results of oat mesocotyl assay of different parts of control and decapitated roots are shown in Fig.45, a. b. c. d. and e.

In the lateral root tips of control plants no IAA was observed (Fig.45.a.), but in the decapitated ones (Fig.45.b.) 1.0 µgm/hgm. IAA eq. was present. Promotor x was 3.56 µgm/hgm. IAA eq. in the control lateral tip which went up to more than 10 µgm/hgm. IAA eq. in decapitated tips. In the control stumps there were more IAA and accelerator x, in comparison to that of decapitated ones. But accelerator α was more (1.4

µgm/hgm. IAA eq.) in decapitated stumps than what was observed in controls (0.04 µgm/hgm. IAA eq. - Fig.45, d. and e.).

It was, however, confirmed that in the absence of tap root tips the lateral tips start to produce more auxin. The results of chromatographic analysis of tap root tips (Fig.45.c.) of control plants again supported the previous observations, that in older roots the main bulk of acid auxins could be found as accelerator x.

The decapitation experiment was however repeated again. On this occasion the plants were allowed to grow for 20 days, and then the acid auxins of lateral tips of both control and decapitated plants were chromatographically analysed. The results (Fig.46.a. and b.) (also Expt. 70.b. in Table 9, Appendix) again showed that decapitation of tap root tips induces promotion of auxin synthesis in lateral tips.

It is again interesting to compare the growth of control and decapitated plants (Table 11). The stumps of decapitated roots have stopped their growth completely but there is no significant difference in total weights of two types of roots. In absence of tips the laterals have grown more vigorously (shown as wts.) and also there is a slight increase in the number of laterals. On the other hand decapitation of root tips did not affect the growth of shoots.

Fig.43. Oat mesocotyl assay of acid and neutral ether fractions of control and decapitated roots (0.5 cm. from the apex excised on 3rd day of sowing) of 9 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. Acid fraction - 30.0 gm. of control roots (with tips) obtained from 9 days' old plants were used in the experiment. Solvent - Isobutanol : Methanol : water.

b. Neutral fraction of (a). Solvent - water.

c. Acid fraction - 30 gm. of decapitated roots from 9 days' old plants were used in the experiment. Solvent - Isobutanol : Methanol : water.

d. Neutral fraction of (c). Solvent - water.

FIG 43

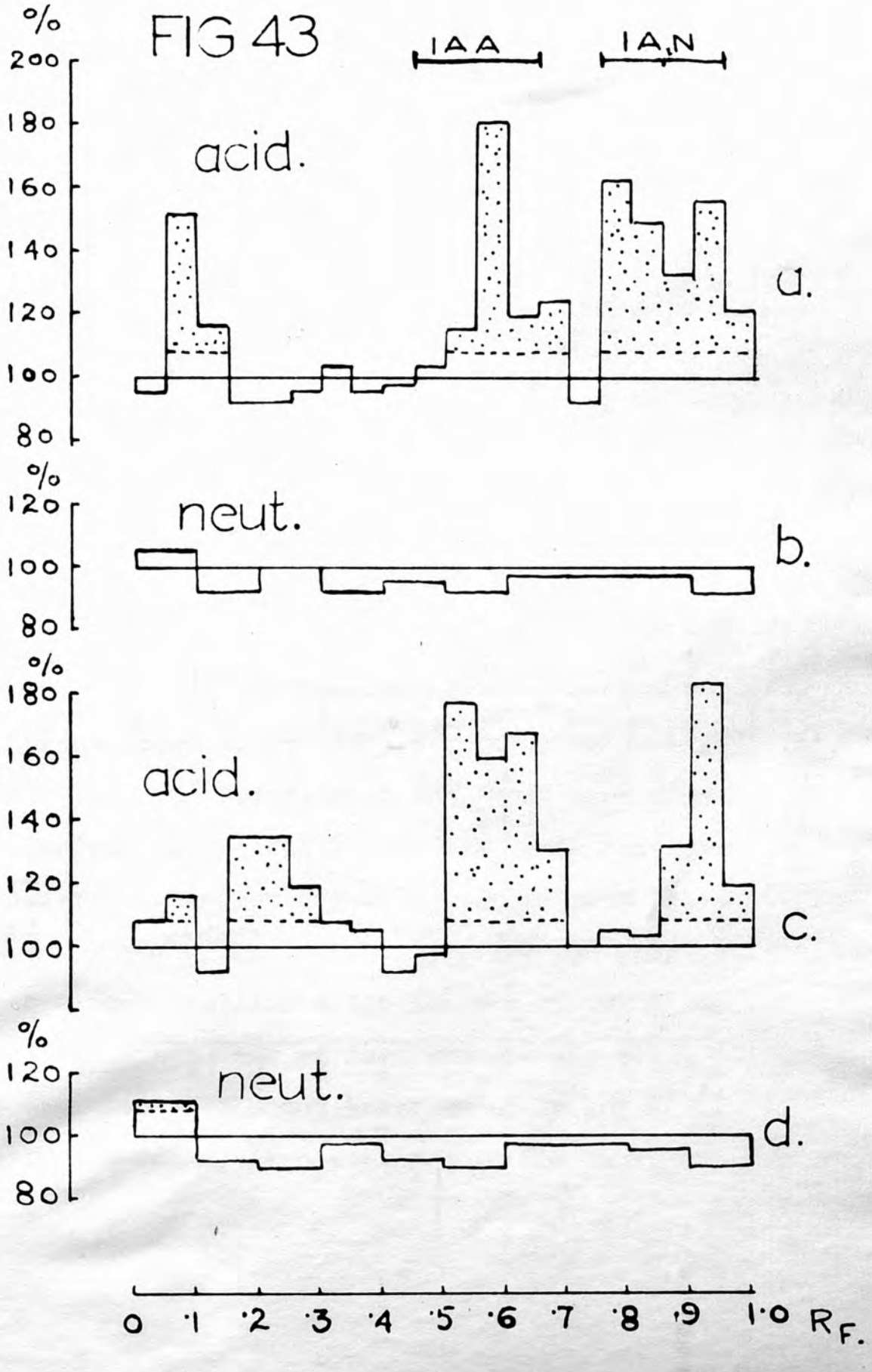

%
200
180
160
140
120
100
80
IAA
IA,N
acid.
a.
%
120
100
80
neut.
b.
%
180
160
140
120
100
acid.
c.
%
120
100
80
neut.
d.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.44. Pea root assay of the acid fractions of control (with root tips) and decapitated (0.5 cm. from the tap root apex, excised on 3rd day of sowing) broad bean plants. (Growth responses plotted against Rf. values.)

a. 30 gm. of control roots obtained from 9 days' old plants were used in the experiment.

b. 30 gm. of decapitated roots obtained from 9 days' old plants were used in the experiment.

FIG 44

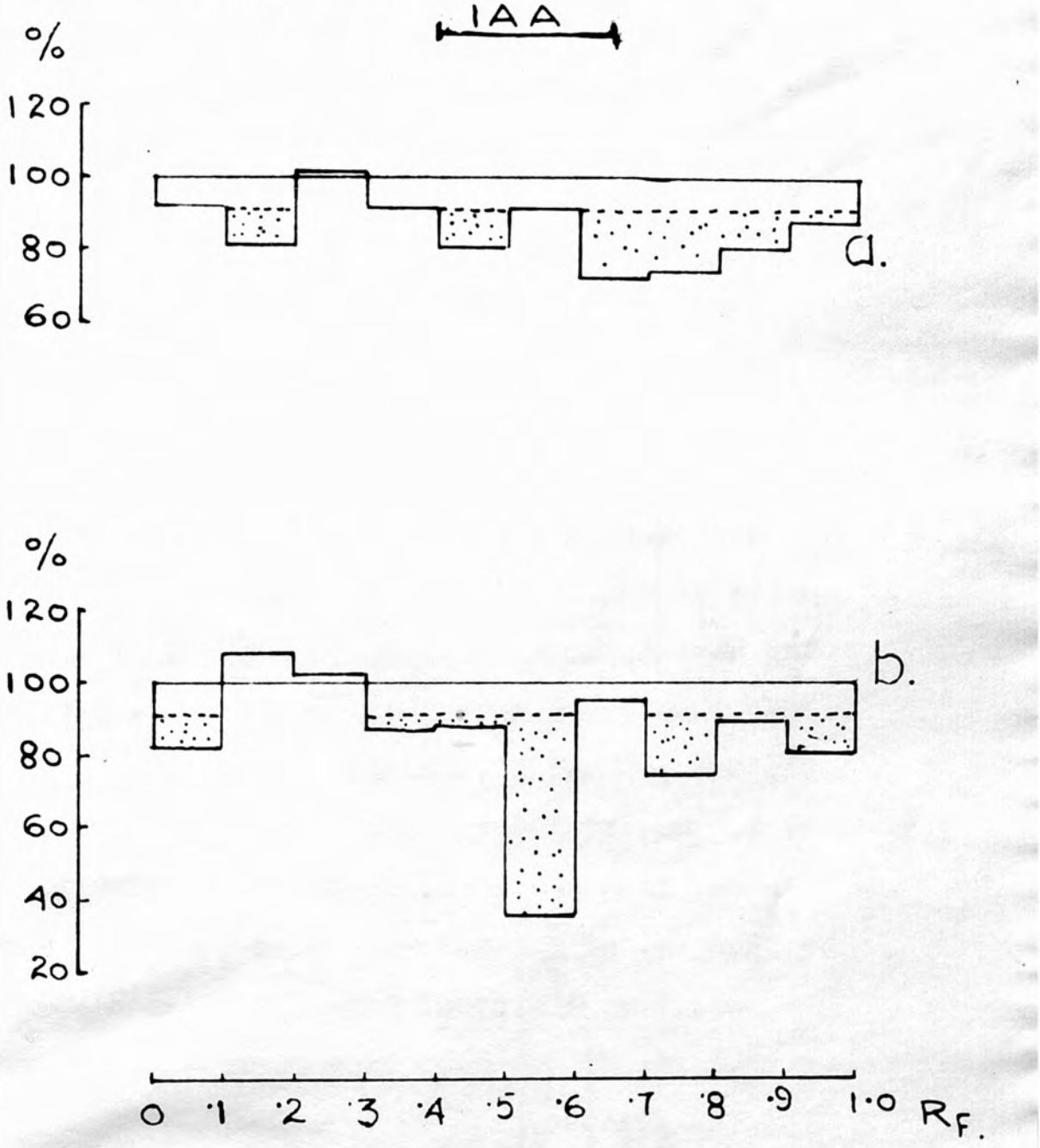


Fig.45. Oat mesocotyl assay of acid fractions of different parts of control and decapitated (0.5 cm. from the tap root apex, excised on 5th day of sowing) broad bean plants (16 days old). (Growth responses plotted against Rf. values.)

a. 1.0 gm. of lateral root tips (300 tips - 0.5 cm. from the apex) from control plants.

b. 1.0 gm. of lateral root tips (310 tips - 0.5 cm. from the apex) from decapitated plants.

c. 0.8 gm. of tap root tips from control plants (72 plants).

FIG 45

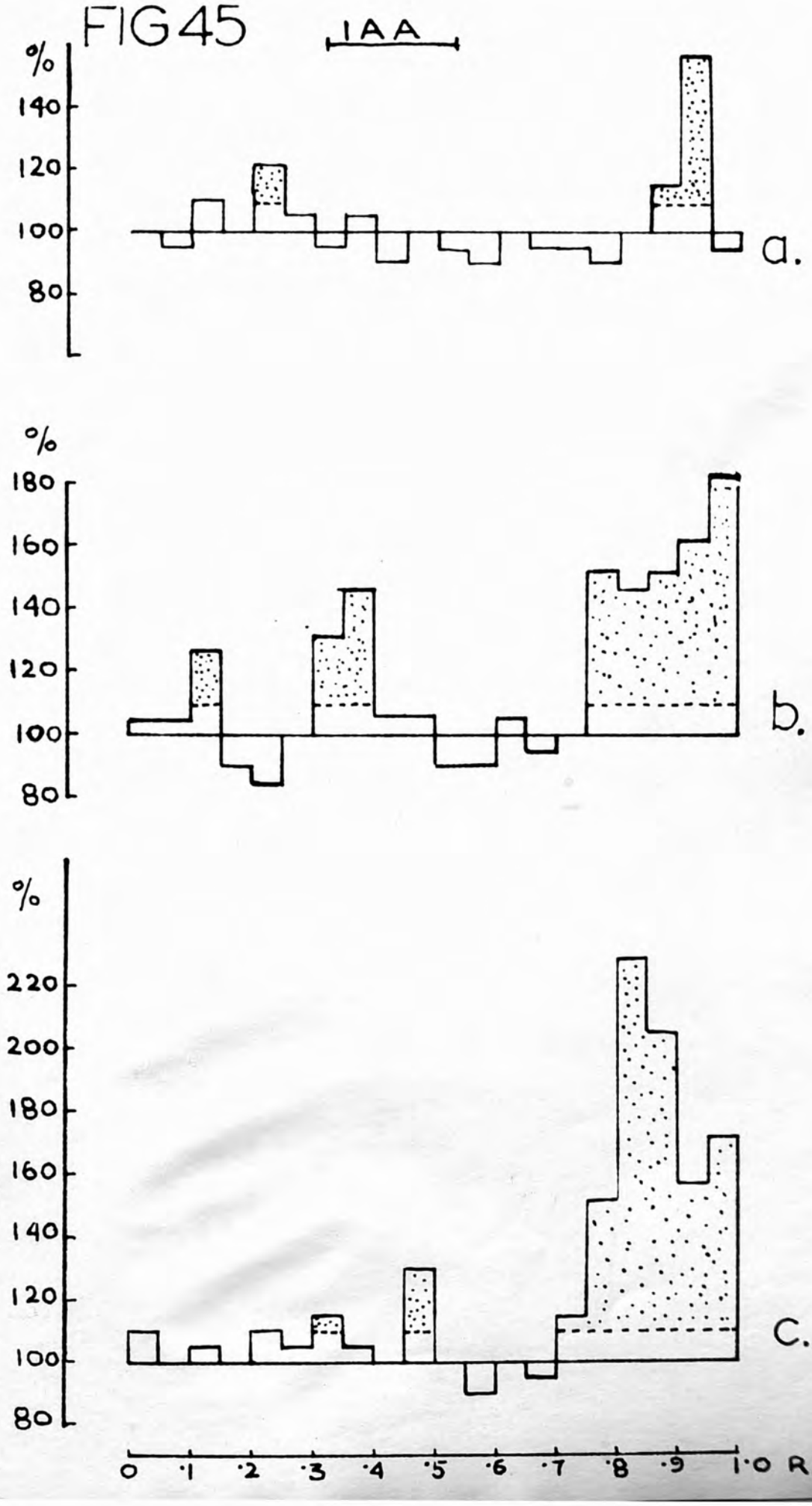

IAA
%
140
120
100
80
a.
%
180
160
140
120
100
80
b.
%
220
200
180
160
140
120
100
80
C.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF

Fig.45. (contd.)

d. 28 gm. of root stumps of decapitated roots (76 stumps).

e. 32.5 gm. of root stumps (50 stumps) of control plants (root tips assayed separately and the results are presented in c.).

FIG 45 (contd.)

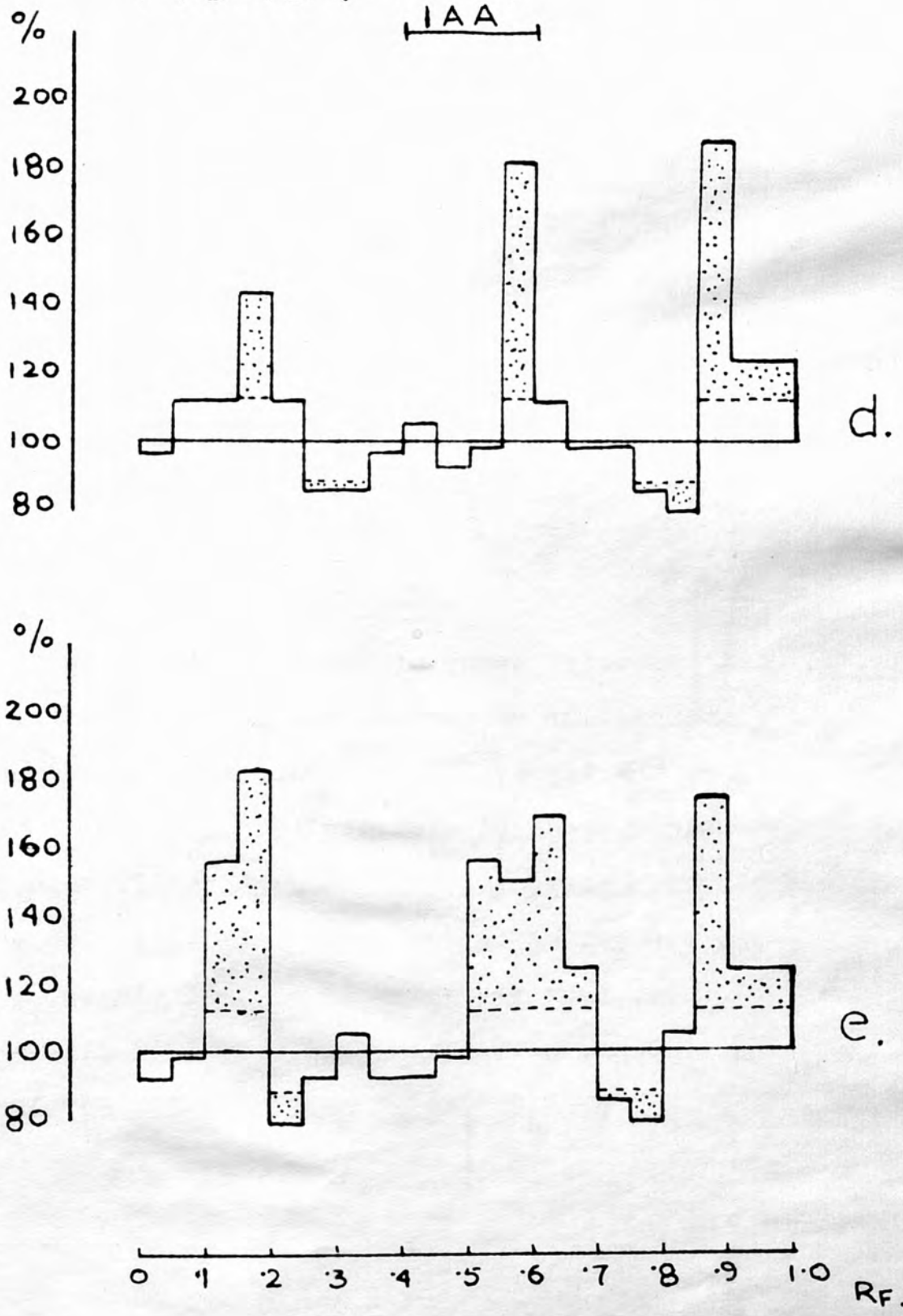

IAA
%
200
180
160
140
120
100
80
d.
%
200
180
160
140
120
100
80
e.
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
RF.

Fig.46. Oat mesocotyl assay of the acid fractions of the lateral tips of control and decapitated (0.5 cm. from the tap-root apex, excised on 3rd day of sowing) broad bean plants (20 days old). (Growth responses plotted against Rf. values.)

a. 1.0 gm. of lateral root tips (312 tips - 0.5 cm. from the apex) of control plants.

b. 1.0 gm. of lateral root tips (310 tips - 0.5 cm. from the apex) from decapitated plants.

FIG 46

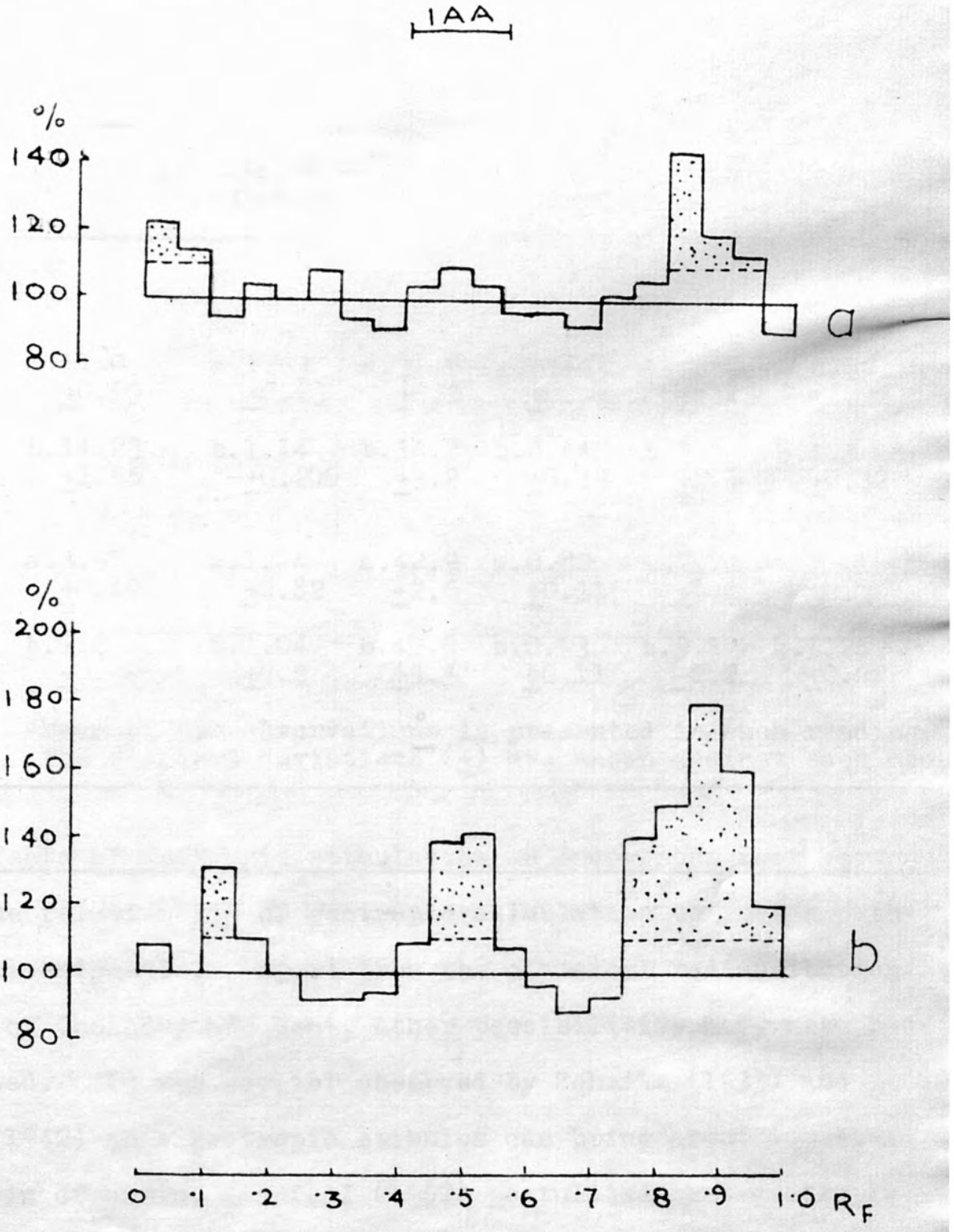

IAA
%
140
120
100
80
a
%
200
180
160
140
120
100
80
b
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
R_F

Table 11.

Root growth of control and decapitated plants (0.5 cm. of root tips being decapitated from 3 days' old plants)*

Treatment.	Total length of root. Cm.	Total wt. of root. gm.	No. of laterals	Wt. of laterals gm.	Length of shoot Cm.	Wt. of shoot. gm.
Final reading on 9th day.						
(i) Control	a.11.09 ±0.99	a.1.03 ±0.23	a.31.7 ±6.3	a.0.425 ±0.08	a.7.58 ±1.46	a.0.96 ±0.22
	b.11.23 ±1.55	b.1.14 ±0.206	b.34.2 ±3.9	b.0.44 ±0.19	b.9.0 ±2.1	b.1.3 ±0.32
(ii) Decapitated plants	a.3.97 ±0.40	a.1.24 ±0.82	a.42.9 ±2.6	a.0.82 ±0.11	a.7.53 ±1.8	a.0.76 ±0.18
	b.2.8 ±0.39	b.1.04 ±0.9	b.40.0 ±3.3	b.0.93 ±0.11	b.9.1 ±2.2	b.1.26 ±0.22

*Mean of ten observations is presented in each reading. The standard deviations (±) are shown against each mean.

(e) Effects of geotropic stimulation on endogenous root hormones

The relationship of geotropic stimulation to root growth is still enigmatic. Apart from the classical redistribution theory of Cholodny and Went, other possibilities have also been suggested. It was earlier observed by Schmitz (1933) and Brian (1942) that geotropic stimulus can bring about increased synthesis of auxin. Rufelt (1957) postulated that geotropic curvature is not due to redistribution of auxin within the

tissue, but due to its increased production in the lower half in relation to the upper half of the root meristem. Again, Audus and Brownbridge (1957) suggested that the geotropic stimulus brings about a de novo synthesis of an inhibitor in the elongating cells of the lower side of the root.

In order to verify the current concepts of geotropism a direct experimental approach was planned. The investigation was pointed towards a study of endogenous auxins in normal and geotropically stimulated root tips with the help of paper chromatographic technique.

The materials used in these investigations were roots of three days' old broad bean seedlings. The roots were subjected to geotropic stimulation on cork mats covered with moist filter paper. Pins were fixed through the cotyledons to hold the seedlings in position on the cork mat. Care was taken not to touch the roots during manipulations and the seedlings were fixed on the cork mat in such a way that the roots did not touch the surface of filter paper. This was done in order to eliminate the effects of touch-stimulation which might complicate the issue.

The drying of roots was avoided by spraying fine jets of distilled water for 10 secs. every 15 mins. from an

approximate distance of one foot from the seedlings. Each cork mat would hold about 15-20 seedlings and it was allowed to stand in a water tank with little distilled water at the bottom. All the seedlings were fixed on the mat in the normal position, with roots pointing downwards and the roots were brought to horizontal position by turning the mat through 90°. The experiments were carried out at room temperature in diffuse light.

In all experiments 0.5 cm. of root tips have been used for extraction and the tissue weight in most experiments was just above 1.0 gm. Only the acid fractions of the extracts have been chromatographically analysed. It may be mentioned here that the roots did not show any curvature even when the roots were stimulated for one hour.

The work began in an attempt to study the auxin content of root tips at different lengths of stimulation time. Samples were collected from control roots and from roots subjected to geotropic stimulation for 15, 30, 35, 40 and 60 mins. Oat mesocotyl test was used to assay the chromatograms. The treated and control extracts were assayed concurrently in each experiment. The results of these experiments have been presented as histograms in Figs. 47(a. and b.) - 51 (a. and b.).

The activity of the substance occurring at IAA zone was determined from the area covered by the substance above the fiducial limit in respective histograms. The relationship of time and percentage stimulation of this substance over control (on gm. wt. basis) has been shown in Fig.53. The activity could not be expressed in IAA equivalents because on many occasions the readings went far above the limits of the calibration curve. The relationship of the time for which roots were stimulated and the increased production of the substance in the IAA zone is further illustrated in Table 12.

Table 12.

Time for Which roots were stimulated. Mins.	Activity in controls. sq.mm. units per gm.wt. of tissue.	Activity in treated. Sq.mm.units per gm.wt. of tissue	% Cont.	Increase in activity over cont.	Increase expressed as % of cont.
15	62.5	74.1	118.5	11.6	18.5
30	39.4	211.5	537.7	182.1	462.1
35	150.0	135.0	900.0	1200.0	800.0
40	93.3	1569.2	1693.6	1475.9	1592.6
60	480.0	975.0	203.1	495.0	103.1

These findings directly prove that geotropic stimulation brings about increased production of a growth substance in the root tips.

In a similar experiment the roots were stimulated for one hour and the acid fractions of both control and treated tips were assayed with pea root section test subsequent to chromatography. The results of this experiment are presented in Fig.52 (a. and b.). A large inhibition at the IAA position of the chromatogram of treated root tips (Fig.52.b.) further emphasised that geotropic responses in roots must be related to synthesis of an inhibitor.

Attempts were made to express the quantities of growth substances in IAA equivalents observed in this and in another one hour experiment where oat mesocotyl assay was used. The results are included in Table 10 (Appendix). In the pea root assay, the stimulated tips showed 142.55 µgm/hgm. IAA eq. of the substance occurring at IAA position, whereas in the control only 0.22 µgm/hgm. IAA eq. was recorded. In oat mesocotyl assay, the concentration of the same substance was 28.33 µgm/hgm. IAA eq. (some readings were possible in the inhibitory concentration range of the calibration curve) in treated root tips and in the control it was 1.03 µgm/hgm. IAA eq. It could be further seen from Fig.52.a. and b. (also Table 10, Appendix) that the amount of inhibitor β showed little or no change in the control and the treated tips.

A close examination of Figs. 47-51 would show that the quantity of substance x gradually fell while the concentrations of the substance at IAA zone went up. The relationship of time of stimulation and gradual disappearance of promotor x is further illustrated in Table 13. When the peak of synthesis was attained at 40 mins. (Fig.50.b.) no trace of other promotors could be observed in the histogram. Later, at 60 mins. (Fig. 51.b.), the substance at IAA zone showed a decline in concentration and other growth substances came into the picture. It would again appear from Fig.53 that the rate of production of this substance proceeded at a very fast rate from 15-40 mins. and the concentration increased exponentially up to this period but there was an abrupt fall to a relatively low value at 60 mins.

Table 13.

Time for which roots were stimulated. Mins.	Activity in controls. sq. mm.units per gm.wt. of tissue	Activity in treated. sq. mm.units per gm.wt. of tissue	% Control
15	211.5	345.8	163.4
30	263.1	11.5	4.3
35	300.0	0	-
40	76.7	0	-
60	375	105	3.6

Regarding the chemical nature of this substance, no conclusive statement can be made. From its position on chromatograms, it is tempting to believe that it is IAA, but in recent investigations (Bennet-Clark et al. 1959) presence of IAA in roots has been doubted. On the basis of present work, it can only be added that in a chromatogram of the acid fraction obtained from 160 gms. of unstimulated roots, positive colour reaction was observed at the IAA region (Rf. 0.45-0.6) afterssprayings with Ehrlich's reagent.

Bennet-Clark and his co-workers (1959) supported the idea of Audus and Brownbridge (1957) regarding the root geotropic mechanisms. But they did not find any difference in the apparent growth substance content in control and stimulated tips. They however suggested that release of material from vacuole to cytoplasm is more probable as a mechanism than production de novo of a growth substance.

It is possible that the production of auxin due to geotropic stimulation (as shown in previous experiments) may be due to release of bound auxins into a free state. In order to investigate this problem free auxins were extracted from control and stimulated root tips (40 mins. presentation time) by ether extraction method (previously described) and the acid fractions were chromatographically analysed using oat mesocotyl assay

(Fig.52, c. and d.). The results supported and confirmed the previous observations that a large amount of auxin is formed in the root tips due to geotropic stimulation (Table 12, appendix). Bound auxins from the same tissues were then extracted (method previously described) and the acid fractions were analysed. The results of oat mesocotyl assay are illustrated as histograms in Fig.52, e. and f.

The amount of substance occurring at the IAA zone in the bound form was 0.007 µgm/gm. IAA eq. in the control and 0.013 µgm/gm. IAA eq. in stimulated tips (Fig.52, e. and f. and Table 12, appendix).

If the increased production of free auxin in stimulated tips was due to a release from bound state, one would expect to find less bound IAA (or the substance occurring at IAA region) in stimulated tips than that in the control. But the experimental results show that the amount of bound IAA in stimulated tips is slightly more than that in the control. Similarly, total amounts of bound acid auxins in control and geo-stimulated tips show slight difference (0.5793 µgm/gm. wt. IAA eq. being observed in control tips and 0.747 µgm/gm. wt. IAA eq. in stimulated tips). On the basis of these observations (see Table 12, Appendix) it will be logical to conclude that the production of the hormone due to geotropic stimulation is in no way connected with the release of bound auxins. The whole

problem naturally resolves into one basic conclusion, that geotropic stimulation triggers off certain enzymatic reactions which bring about increased synthesis of the auxin occurring at the IAA zone.

After being convinced that problems related to geotropism were closely related to synthesis of auxin, attempts were made to re-investigate the distribution of growth substances on upper and lower halves of stimulated root tips.

The roots were subjected to geotropic stimulation for an hour and the upper and lower halves of 0.5 cm. root tips were halved with the help of a sharp thin blade. Oat mesocotyl assay was used to analyse the acid fractions of extracts. By careful adjustment of timing, the same period of stimulation was ensured for all the roots.

The results of two similar experiments performed on separate occasions have been presented in Fig.54, a. b. c. and d. In the first experiment 0.5 gm. of upper and 0.48 gm. of lower half was obtained from 101 tips (Fig.54. a. and b.) In the second experiment weights of both upper and lower halves were 0.5 gm. and 104 root tips were used in this case. (Fig.54, c. and d.)

Quantities of growth active compounds were determined in IAA equivalents and the results are shown in Table 11 (Appendix).

In both experiments more IAA as well as total acid auxins were found to be present in the upper half of the roots. However, a relatively higher amount of promotor x was recorded in the lower half of the roots in both experiments (Fig.54, and Table 11, Appendix).

The results of these experiments do not, however, support the redistribution theory. Ching and Fang (1958) stated from their investigations on in vivo distribution of administered radioactive IAA (IAA-1-C^{14}) in geotropically stimulated organs, that no indication of unequal distribution of auxin could be established. Considering the diverse fate of administered IAA within the tissue, the authors (Ching and Fang) admitted that their results will be useful only for the purpose of speculation.

Fig.47. Histograms illustrating the oat mesocotyl assay results of chromatograms of acid auxins from control (unstimulated) and geotropically stimulated (for 15 mins.) root tips (0.5 cm. from the apex) of 3 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. 1.3 gm. of root apex material (110 root tips) from control plants.

b. 1.2 gm. of root apex material (112 root tips) from stimulated plants.

FIG 47

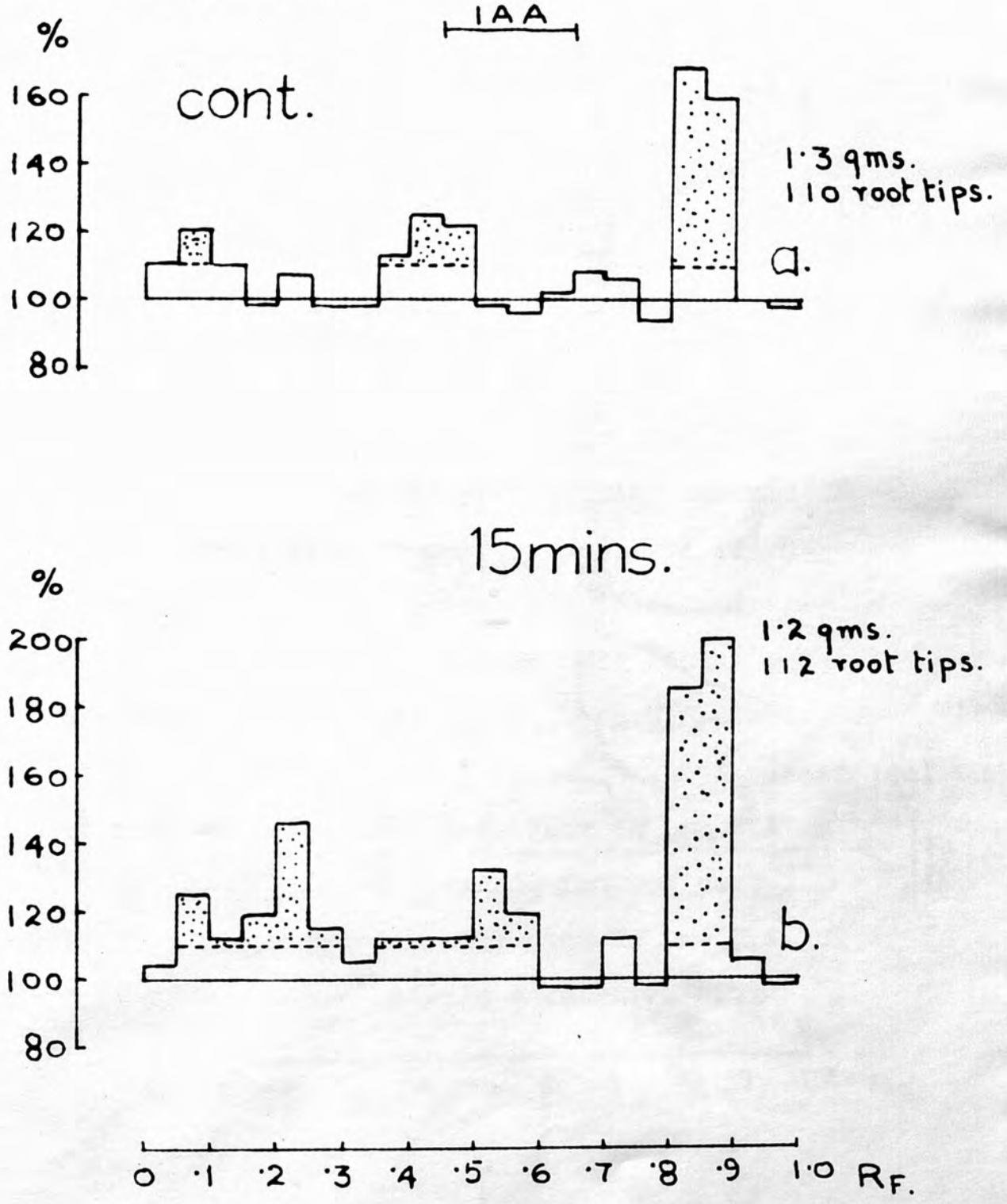

%
160
140
120
100
80
cont.
IAA
1·3 gms.
110 root tips.
a.
15mins.
%
200
180
160
140
120
100
80
1·2 gms.
112 root tips.
b.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.48. Histograms illustrating the oat mesocotyl assay results of chromatograms of acid auxins from control (unstimulated) and geotropically stimulated (for 30 mins.) root tips (0.5 cm. from the apex) of 3 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. 1.9 gm. of root apex material (125 root tips) from control plants.

b. 1.3 gm. of root apex material (113 root tips) from stimulated plants.

FIG. 48.

cont.

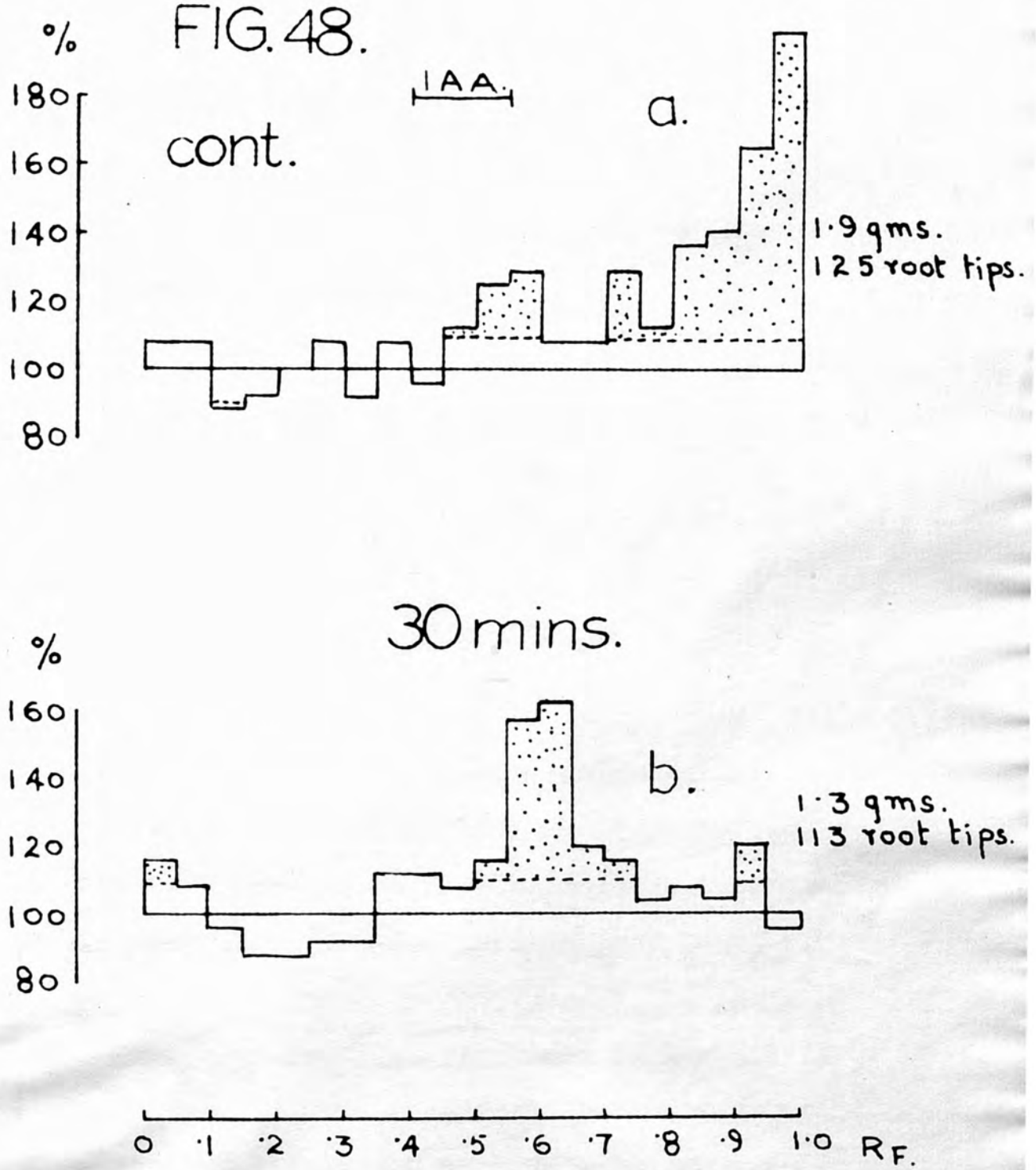

%
180
160
140
120
100
80
IAA.
a.
1·9 gms.
125 root tips.
30 mins.
%
160
140
120
100
80
b.
1·3 gms.
113 root tips.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.49. Histograms illustrating the oat mesocotyl assay results of chromatograms of acid auxins from control (unstimulated) and geotropically stimulated (for 35 mins.) root tips (0.5 cm. from the apex) of 3 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. 1.0 gm. of root apex material (105 root tips) from control plants.

b. 1.0 gm. of root apex material (108 root tips) from stimulated plants.

FIG.49.

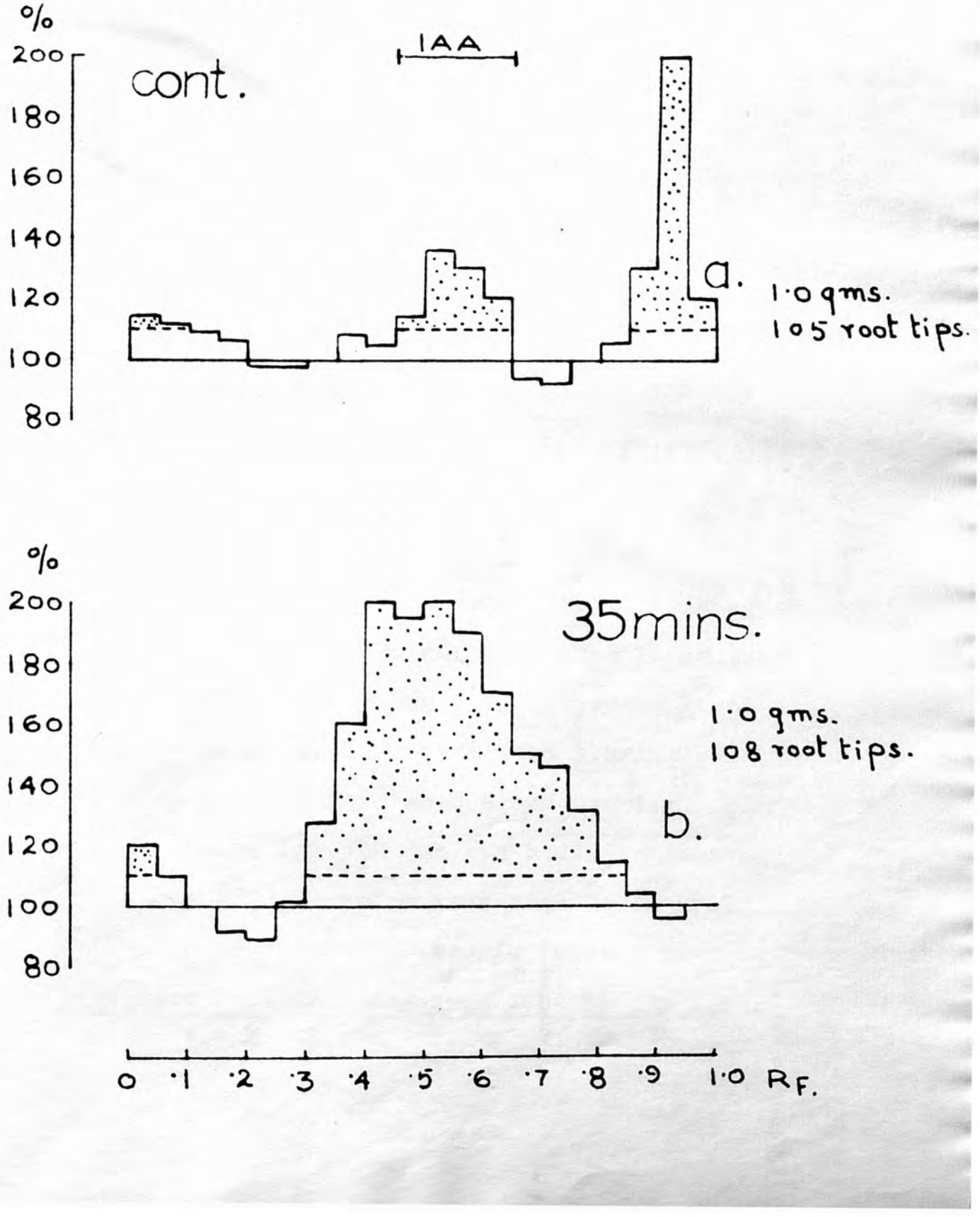

%
200
180
160
140
120
100
80
cont.
IAA
a.
1·0 gms.
105 root tips.
%
200
180
160
140
120
100
80
35mins.
1·0 gms.
108 root tips.
b.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.50. Histograms illustrating the oat mesocotyl assay results of chromatograms of acid auxins from control (unstimulated) and geotropically stimulated (for 40 mins.) root tips (0.5 cm. from the apex) of 3 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. 1.5 gm. of root apex material (118 root tips) from control plants.

b. 1.3 gm. of root apex material (120 root tips) from stimulated plants.

FIG. 50.

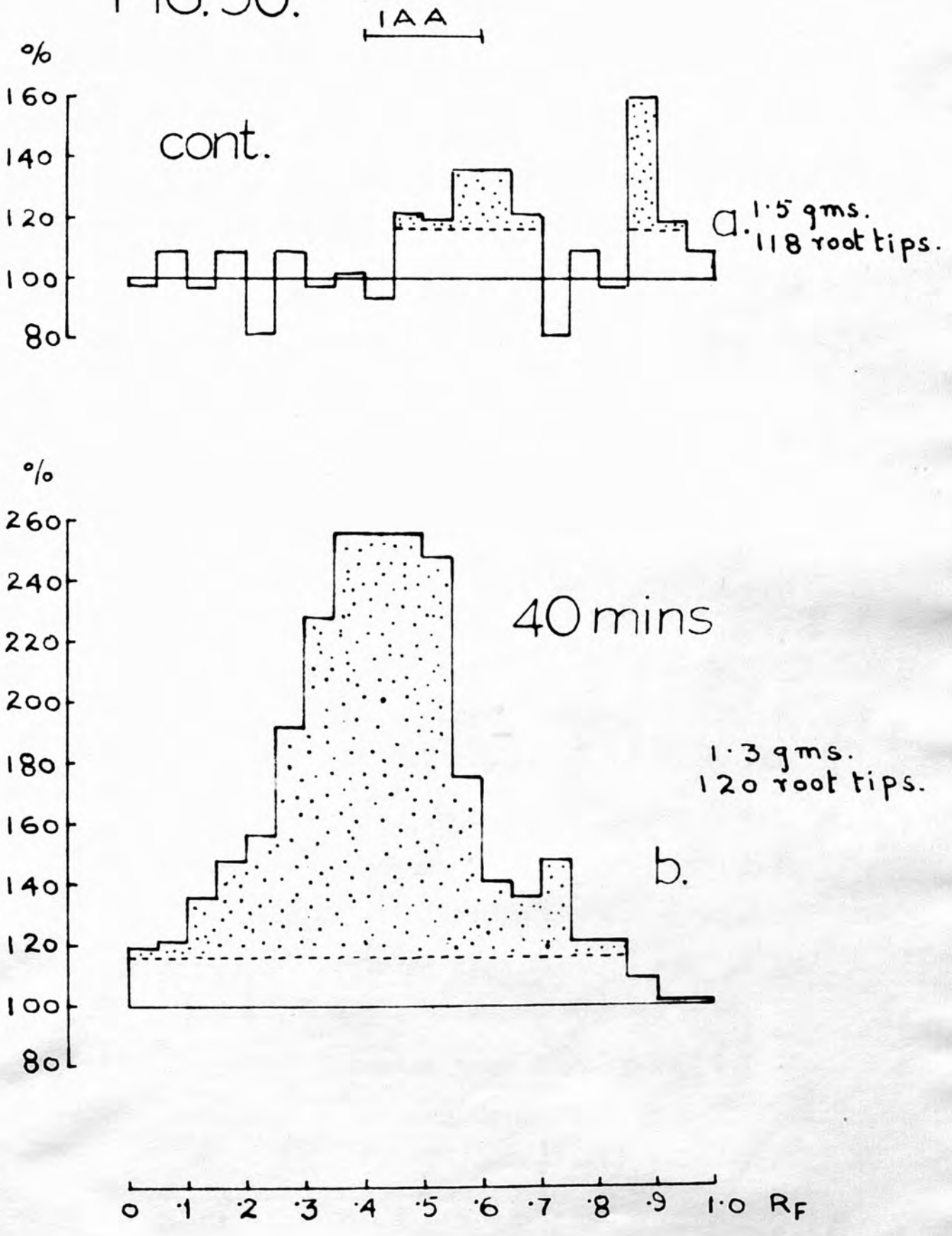

IAA
%
160
140
120
100
80
cont.
a.
1·5 gms.
118 root tips.
%
260
240
220
200
180
160
140
120
100
80
40 mins
1·3 gms.
120 root tips.
b.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF

Fig.51. Histograms illustrating the oat mesocotyl assay results of chromatograms of acid auxins from control (unstimulated) and geotropically stimulated (for 60 mins.) root tips (0.5 cm. from the apex) of 3 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. 1.0 gm. of root apex material (110 root tips) from control plants.

b. 1.0 gm. of root apex material (115 root tips) from stimulated plants.

FIG 51

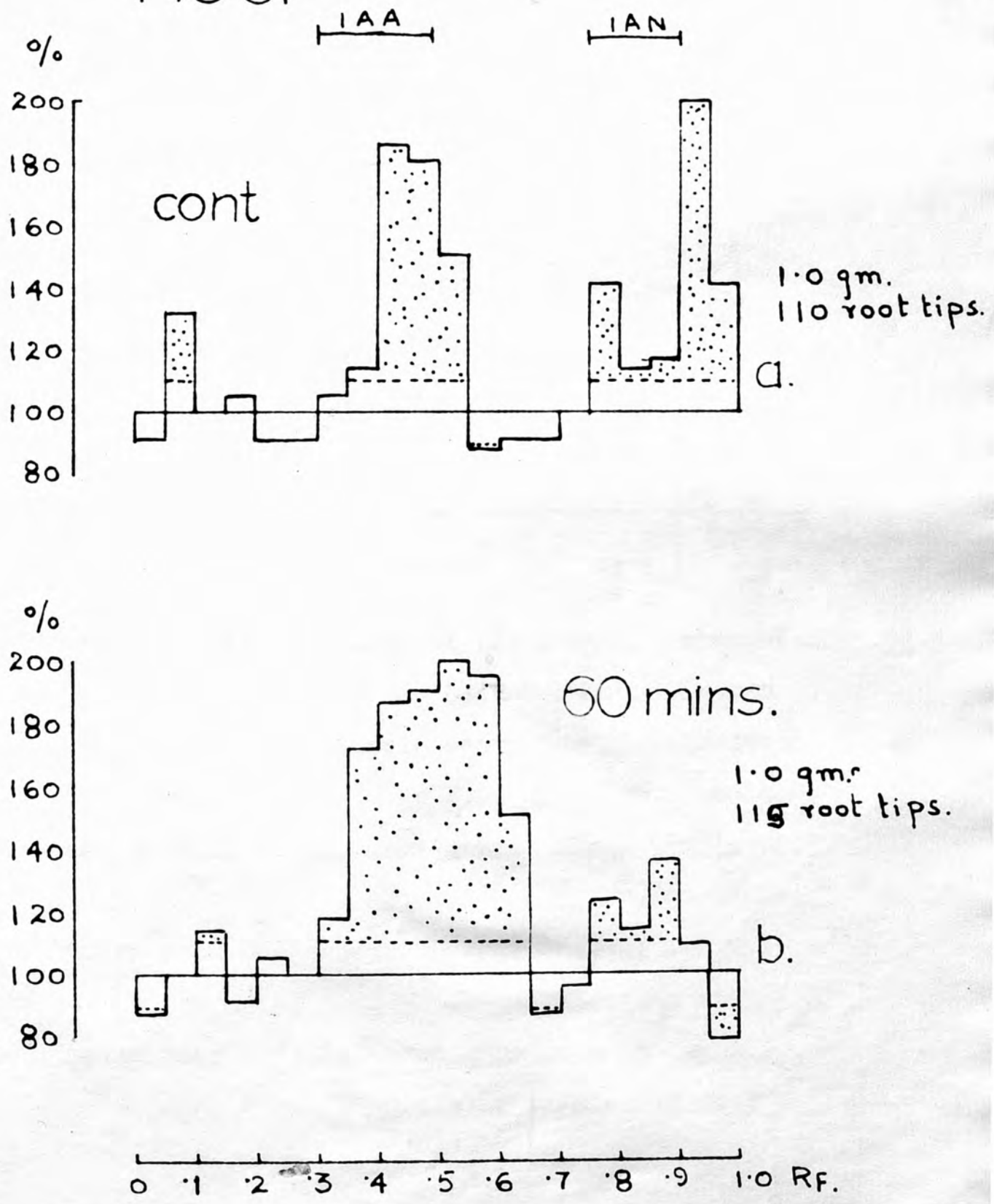

IAA
IAN
%
200
180
160
140
120
100
80
cont
1·0 gm.
110 root tips.
a.
%
200
180
160
140
120
100
80
60 mins.
1·0 gm.
115 root tips.
b.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 Rf.

Fig.52. Histograms illustrating the pea root segment assay of chromatograms of acid auxins from control (unstimulated) and geotropically stimulated (for 60 mins.) root tips (0.5 cm. from the apex) of 3 days' old broad bean plants. (Growth responses plotted against Rf. values.)

a. 1.2 gm. of root apex material (108 root tips) from control plants.

b. 1.1 gm. of root apex material (106 root tips) from stimulated plants.

FIG 52

cont.

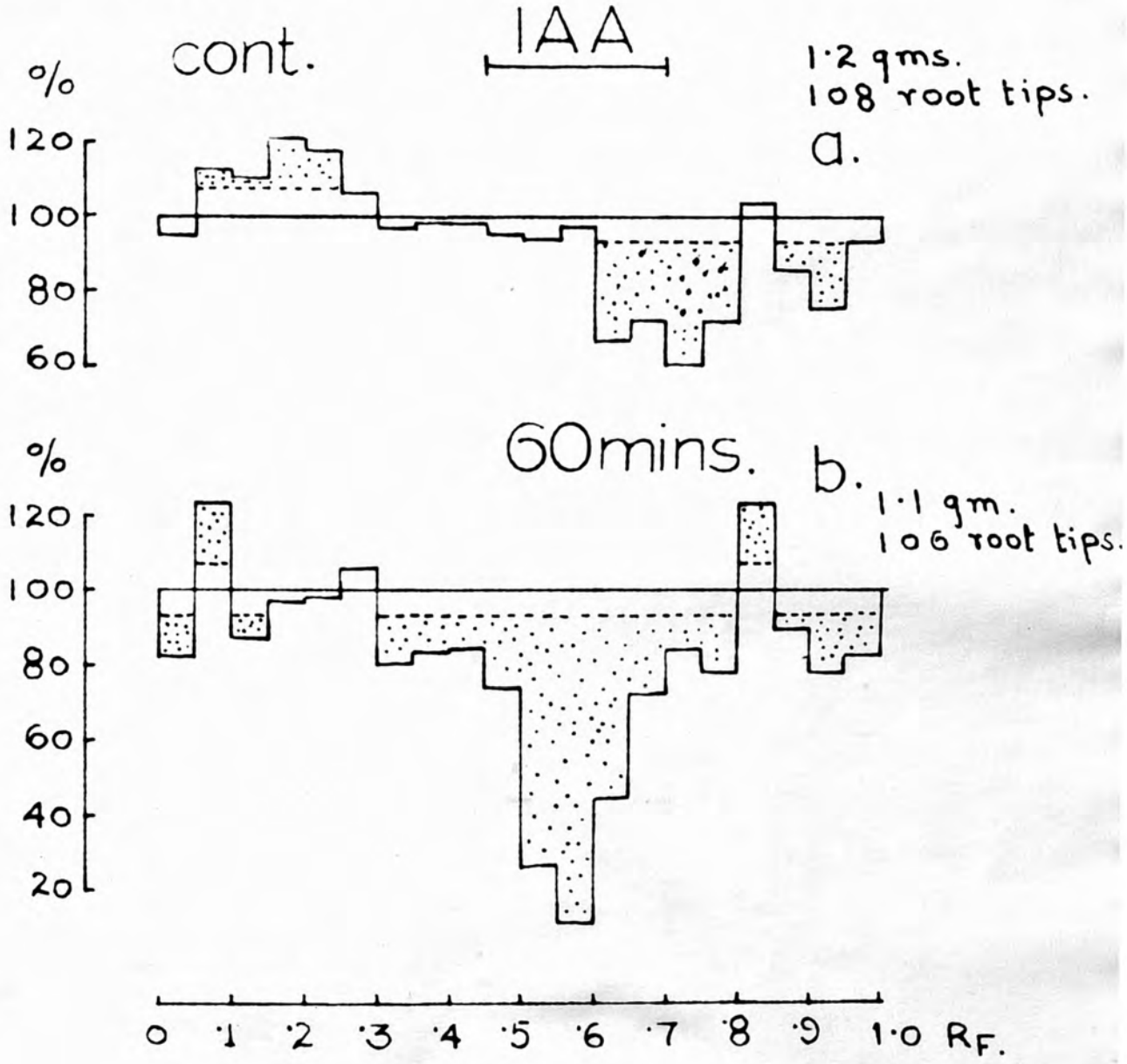

IAA
%
120
100
80
60
1·2 gms.
108 root tips.
a.
60mins.
b.
%
120
100
80
60
40
20
1·1 gm.
106 root tips.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.52. (contd.)

c. Free acid auxins (by ether extraction method) from control (1.3 gm. of root apex material - 112 root tips) root tips.

d. Free acid auxins (by ether extraction method) from geotropically stimulated (40 mins.) root tips (1.1 gm. of root apex material - 110 root tips).

FIG 52 (contd.)

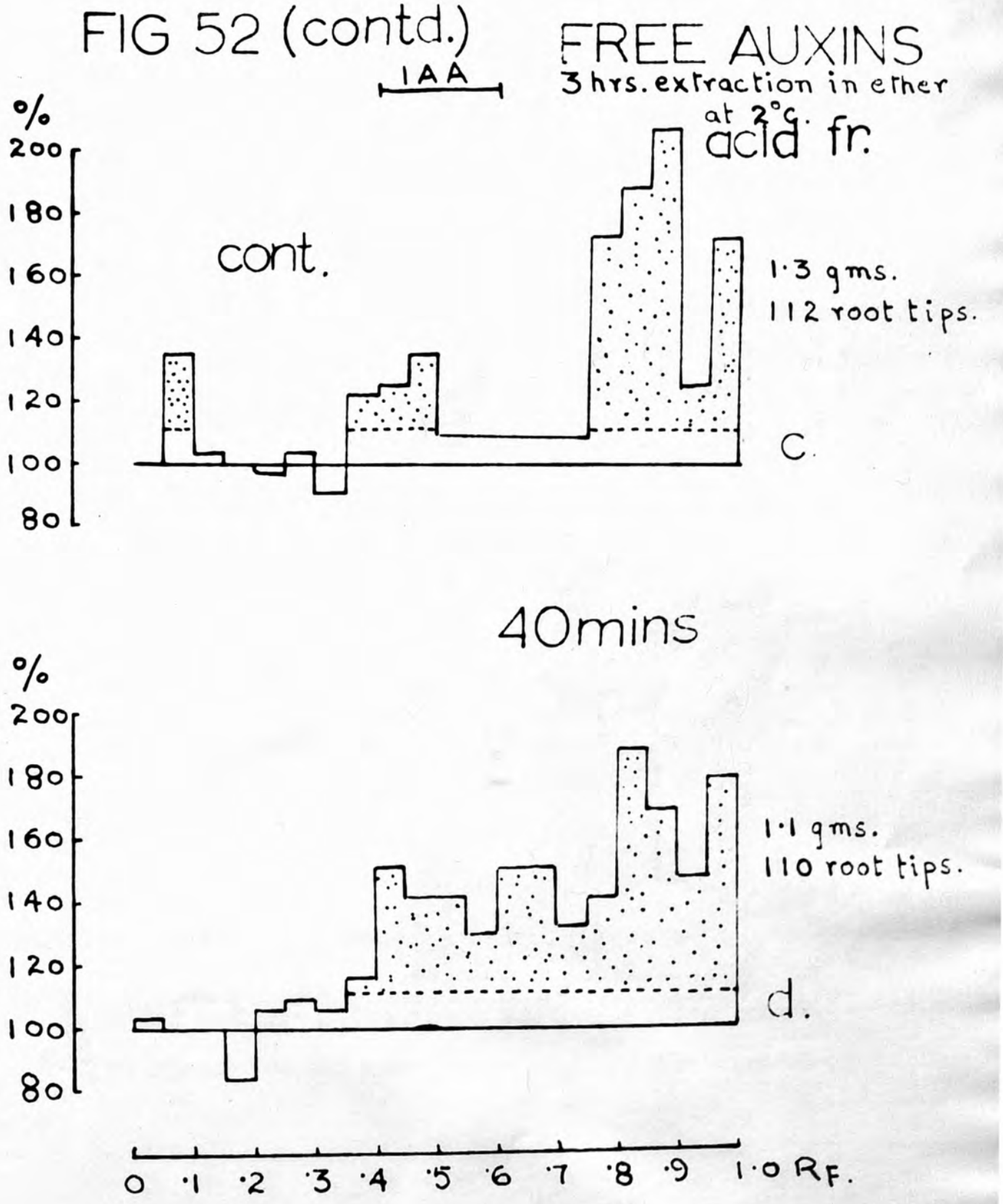

FREE AUXINS
3 hrs. extraction in ether at 2°c.
acid fr.
IAA
%
200
180
160
140
120
100
80
cont.
1·3 gms.
112 root tips.
c.
40mins
%
200
180
160
140
120
100
80
1·1 gms.
110 root tips.
d.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

Fig.52. (contd.)

(Growth responses plotted against Rf. values.)

e. Bound acid auxins in the same tissue as in (c).

f. Bound acid auxins in the same tissue as in (d).

FIG 52 (contd.)

Bound auxin
(after extraction of free auxin)
cont.

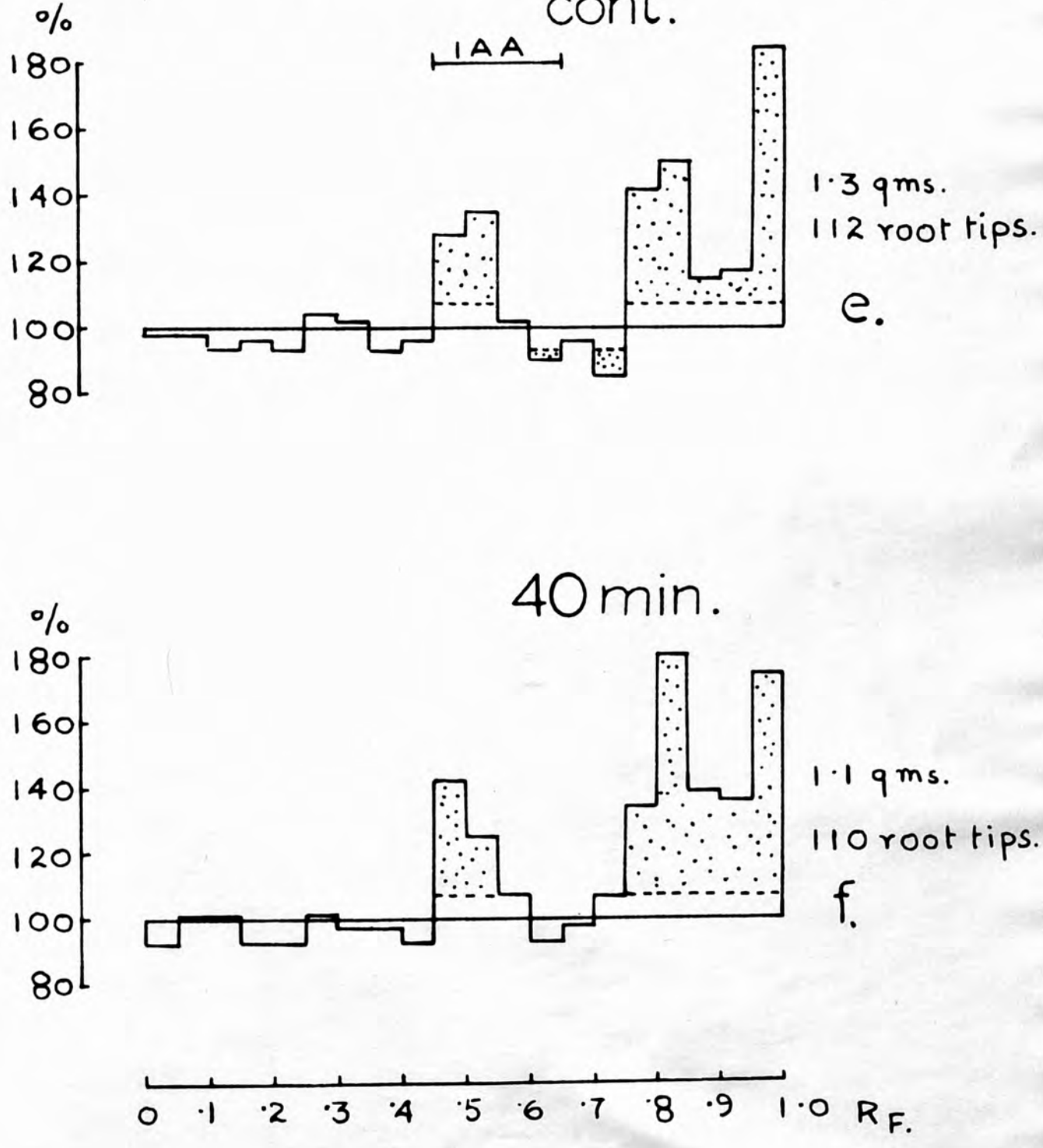


Fig.53. Relationship between geotropic stimulation (presentation time in mins.) and synthesis (expressed as % stimulation over control) of the growth substance occurring at the IAA region of chromatograms. (For further explanation see text.)

FIG. 53.

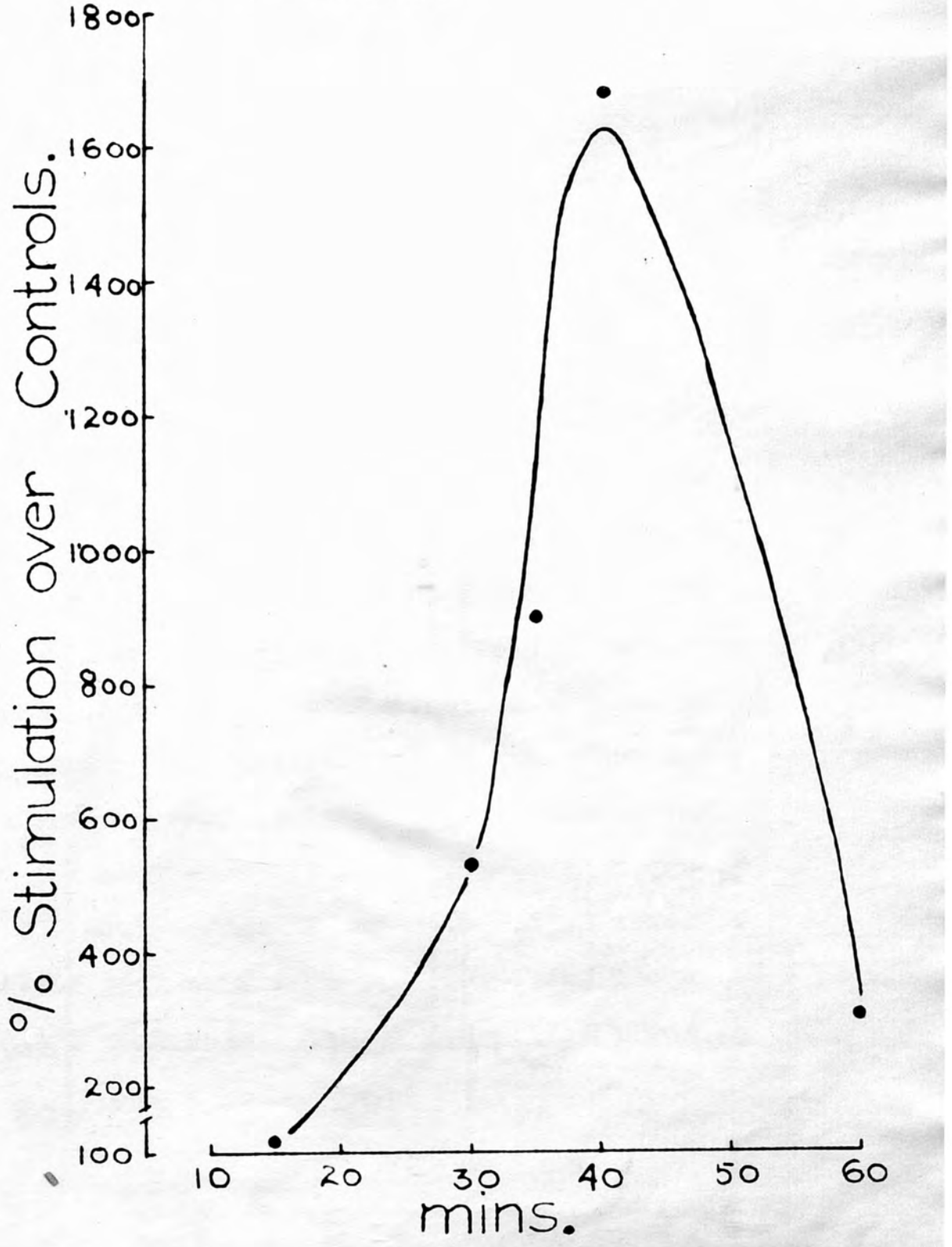

% Stimulation over Controls.
1800
1600
1400
1200
1000
800
600
400
200
100
10
20
30
40
50
60
mins.

Fig.54. Histograms illustrating the distribution of acid growth substances in the upper and lower halves of the tips (0.5 cm. from the apex) of horizontally (for 60 mins.) placed broad bean roots. Results obtained by oat mesocotyl assay of chromatograms. (Growth substances plotted against Rf. values.)

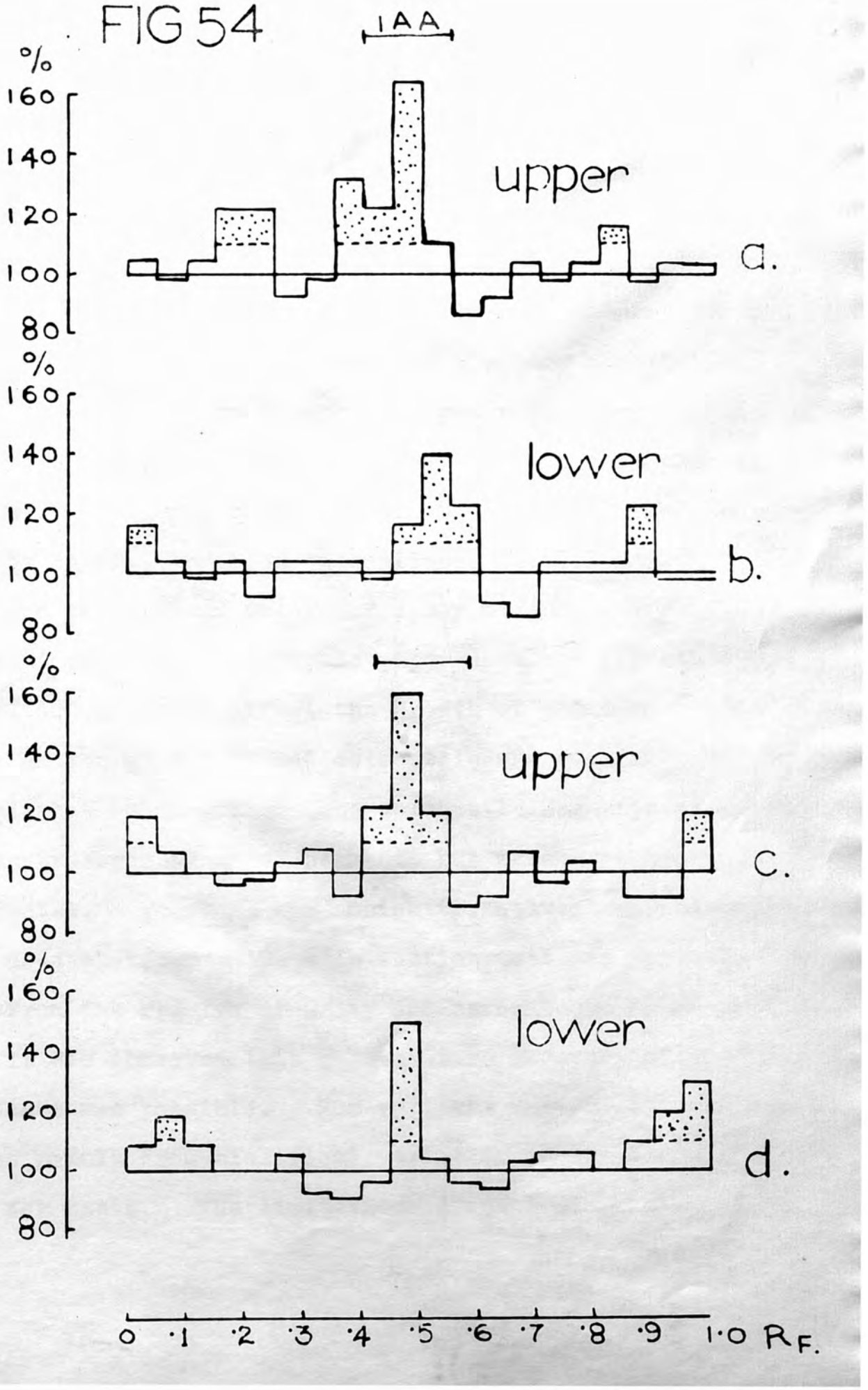
a. upper half. 0.5 gm. of tissue from 101 tips.

b. lower half. 0.48 gm. of tissue from 101 tips.

c. upper half. 0.5 gm. of tissue from 104 tips.

d. lower half. 0.5 gm. of tissue from 104 tips.

FIG 54


IAA
%
160
140
120
100
80
upper
a.
%
160
140
120
100
80
lower
b.
%
160
140
120
100
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upper
c.
%
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lower
d.
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 RF.

C H A P T E R I V.

DISCUSSION

The earlier investigations of this thesis are devoted to a study of growth conditions of bioassay materials. Attempts were made to improve the pea root assay method devised by Audus and Thresh (1953), without any definite success. However, useful information was obtained about effects of age, location of sections, initial length of sections and optimum volume of assay medium. It was further observed that chromatographic paper does not contain any inhibitor which can affect the growth of sections. Experiments on the growth of oat coleoptile and oat mesocotyl showed that on many occasions coleoptile segments grow less in the presence of paper segments but mesocotyl growth is unaffected. To avoid any ambiguity, solvent washed papers were used wherever coleoptile section test was applied.

From the results of assay and chromatography of synthetic IAA, it was observed that quantitative recovery of the substance was possible. However, the variability of results arises mainly from biological variation of the tissue segments used for assay. The limitations of biological variability

could be minimised to a great extent by repetition of experiments. Colorimetric study on the recovery of IAA in preliminary and later experiments showed that loss of the substance due to chromatography and elution was insignificant. While working with plant extracts, other causes of variations could be due to the occurrence of variable amounts of growth substances in the tissue itself, and variations in extraction efficiency.

The general practice for quantitative determination of endogenous growth substances (i.e. from standard IAA calibration curves) can be justly criticised on the following grounds:

a. Variability of response of assay material towards IAA.

b. On the basis that the unknown compounds separated by chromatographic method have, in all possibility, different concentration-response curves.

So long as the chemical nature of the endogenous growth substances is not established, it is not possible to determine their absolute amounts on the basis of IAA-calibration curve. It was frequently noticed in the course of this investigation that accelerator x showed responses which were beyond the limits of the standard curve prepared from repeated experiments. Again, in experiments on geotropism, it was observed that the amount of the substance occurring at the

IAA zone of the chromatogram could not be expressed on the basis of calibration curve.

However, attempts have been made in this investigation to obtain a quantitative expression of the growth substances in terms of IAA equivalents. Nevertheless, it must be stated that these quantities are nothing but a basis for comparison, rather than the absolute amounts present in the tissue.

Problems related to extraction and purification of growth substances have also been investigated in the earlier sections of this investigation. The statement that different qualitative and quantitative pictures of endogenous auxins could be obtained by following different methods of extraction (Bentley, 1958) has been found to be true.

From investigations on growth substances of pea roots separated by purification through acetonitrile and hexane, one promotor at low Rf. value and four inhibitors were detected. Pea root section test was applied for bioassay of chromatograms. One of these inhibitors occurred at the same Rf. value as that of synthetic IAA. The substance occurring at an Rf. value lower than IAA has been found to occur in numerous tissues and also in pea roots (Kefford, 1955; Audus and Thresh, 1956; Audus and Gunning, 1958).

This substance which acts as promotor in both root and shoot assays was named as accelerator ∝ by Bennet-Clark and Kefford (1953). Stowe and Thimann (1954) thought that this substance is identical to indolepyruvic acid. Housley and Bentley (1956), however, interpreted that this was an artefact produced by bicarbonate treatment. Audus and Gunning (1958) suggested that occurrence of this substance where bicarbonate purification was not applied (i.e. acetonitrile purification method), could be due to indoleacetylaspartic acid (Good et al., 1956). [illegible] On the basis of biological properties of indole-pyruvic acid (Bentley, 1958; with reference to the work by Bentley, Farrar, Housley, Smith and Taylor, 1956), it was suggested that accelerator ∝ cannot be identical to indole-pyruvic acid. However, from present investigations by pea root assay method, this promotor can be related to indole-aspartile acetic acid, because alkali and heat treatment was omitted in the extraction method (similar reasoning offered by Audus and Gunning, 1958).

Of the other three inhibitors, one (Rf. 0.35-0.5) could be related to inhibitor β. Audus and Thresh (1956) detected this in the acid fraction of the pea root extract, and Audus

and Gunning (1958), following acetonitrile method, also observed inhibition of wheat coleoptile sections with almost the same Rf. values. Audus and Gunning found interference due to inhibitor and promotors in their assays above this Rf. value, but in the present test two inhibitors (Rf. 0.6-0.7 and 0.8-1.0) could be detected. The substance travelling near the solvent front (Rf. 0.8-1.0) was also found to occur in the acid fraction of extracts (Audus and Thresh, 1956). There were also indications of the occurrence of a promotor at Rf. 0.85-0.9.

When the investigation was extended towards the study of acetonitrile soluble substances of broad bean roots, four promotors were detected, by oat coleoptile and mesocotyl assays. (Figs. 10 and 11) One of the promotors occurred at IAA position of chromatograms and the other just before it (accelerator α). Kefford (1955) also found these two substances in the acid fraction of broad bean roots. But, in acetonitrile method, no trace of inhibitor could be found from the results of oat coleoptile section test. However, when the acid fraction of the broad bean root extract was chromatographically analysed using the oat coleoptile assay, the picture (Fig.28.a.) of endogenous growth substance was identical to that observed by Kefford. From later

experiments on water soluble growth substances, it was observed that some of these compounds are soluble in acetonitrile (Fig.55.b.). It would appear that acetonitrile soluble growth substances which are found in pea roots and broad bean roots are coming from both acid and water soluble fractions, and it would be difficult to identify them only on the basis of Rf. values.

When it was found out from a parallel investigation carried out in this laboratory (Audus and Gunning, 1958) that acetonitrile method is not suitable for quantitative work, experiments were carried out to explore the possibilities of purification by water chromatography. In consideration of the serious disadvantages of this method (mentioned before), it was not adopted as the standard technique. However, it may be mentioned that in the water chromatographic method three inhibitors in pea root extract were detected by pea root assay, whereas four inhibitors were found to occur in chromatograms where acetonitrile method was applied (Fig.12.a. and c.).

Moreover, less IAA was found to be present where the acetonitrile method was applied than that observed in the water chromatographic method. However, in both cases the same quantities of root tissues were used for extraction.

Finally, the fractionation method (described under

Material and Methods) was adopted for purification purposes and growth substances occurring in each fraction were separately analysed.

It would be appropriate to discuss here the nature of the growth substances obtained in the acid fraction of broad bean root extracts. It has been clearly shown in this investigation that all the growth substances occurring in this fraction could not be detected by a single assay method. The following table shows the activities of the acid-growth substances in different assay methods:

Table 14.
(↑ = Promotion; ↓ = Inhibition.)

Assay	Accelerator α Rf. around 0.1	IAA Rf. around 0.5	Inhibitor β Rf. around 0.8	Accelerator x Rf. around 0.9
Oat mesocotyl	↑	↑	very little or no response	↑
Oat coleoptile	↑	↑	↓	masked by inhibitor .
Wheat Coleoptile	↑	↑	↓	"
Pea root	↑	↓	↓	" (Slight promotion of growth observed in one experiment)

From repetition of experiments it was observed that Rf. values of these substances vary considerably, but their relative

positions are always constant. These fluctuations of Rf. values are surely related to temperature variations of the room where the chromatographic chambers were kept.

From dilution tests it was observed that the growth effects of the endogenous growth substances take place within a narrow range of concentration; slight change in concentration could bring about large differences in growth activity. On the basis of present investigation, no hasty prediction about the chemical nature of these substances could be made. Although visible colour reaction was obtained at the IAA region of chromatogram containing acid ether fraction, no conclusive deductions can be made. From recent investigations on endogenous auxins of root apex material of Vicia faba, Bennet-Clark and his co-workers (1959) stated that IAA is present either in very low concentration or is absent in the tissue. They however found other material with Rf. near that of IAA to be present in large quantities (about 1 p.p.m. IAA eq.). They further detected the presence of a volatile "growth substance".

Potassium permanganate spraying produced a gray spot at 0.7-0.95 Rf. value (position of substance x) showing the presence of organic acids, but Ehrlich's reagent did not produce any colour reactions in this region. These observations support

the results of Housley and Taylor (1958) and confirm that growth activities in inhibitor β zone could be attributed to organic acids. The previous authors observed that inhibitor of potato tuber peel contains a complex mixture of aliphatic acids. From observations made in the course of this investigation, it can be stated that one of the constituents of the complex organic acids occurring at higher Rf. values than IAA produces consistent growth promotion to oat mesocotyl sections. Its expression was masked in pea root, oat and wheat coleoptile assays by the inhibitory effect of the other substance (or substances) occurring at almost the same Rf. values. Another possibility is that inhibitor β and promotor x are identical substances showing different growth effects in different assays.

The picture of acid auxins of root tissues (whole root) at different ages of broad bean plants presented an interesting picture. It was observed that the roots increased in length up to 12 days and then the growth was stopped. The total acid auxin of the roots showed slight increase from 3 to 12 days and then there was a steady fall up to the 25th day (Fig.22). The substance occurring at IAA region of chromatograms also presented a similar lowering of concentration with ageing. Accelerator α and accelerator x (Fig.23) presented considerable

shifting of concentrations in the initial stages of growth, but later (from the 18th day onwards) there were clear indications of gradual lowering of concentrations of both substances as age increased. In brief, concentrations of individual acid auxins fall with age. It was highly improbable that this effect was due to starvation, because the shoots continued healthy and vigorous growth long after the 25th day.

The initial rise in concentration of total auxin and IAA could be related to development of lateral meristems which become visible about the same time (9th day). The growth of the laterals (recorded as changes in weight) continues up to the 15th day and then the growth stops (Fig.25.a.). At this stage (9-15 days) the appearance of additional meristems and increased production of auxin keeps the total auxin content to the root to a higher level. But later, from the 15th day, the growth of tap roots as well as the laterals stops, and the endogenous level of auxins gradually starts to come down.

It naturally follows that decline in growth rate of the older roots was correlated not with high concentration of endogenous auxins but with gradual disappearance or inactivation of growth substances. Moewus and Moewus (1952) also observed in cress roots that auxin content of older roots has about half of that present in shorter roots.

FIG 55

Fig. 55. Relationship of root growth and endogenous auxin content as presented by Pilet (1951).

Variations in development and content of root-auxins (a) as a function of the time (T).

GT = normal growth curve.

A = the curve for auxin content of the root of Lens.

O-M = the quantity of auxin is suboptimal.

M-N = the quantity of auxin increases as growth increases.

From N the concentration of auxin becomes too strong and growth falls. Above N this concentration is clearly supraoptimal and consequently development is inhibited.

Fig.55. Relationship of root growth and endogenous auxin content as presented by Pilet (1951).

Variations in development and content of root-auxins (a) as a function of the time (T).

CT = normal growth curve.

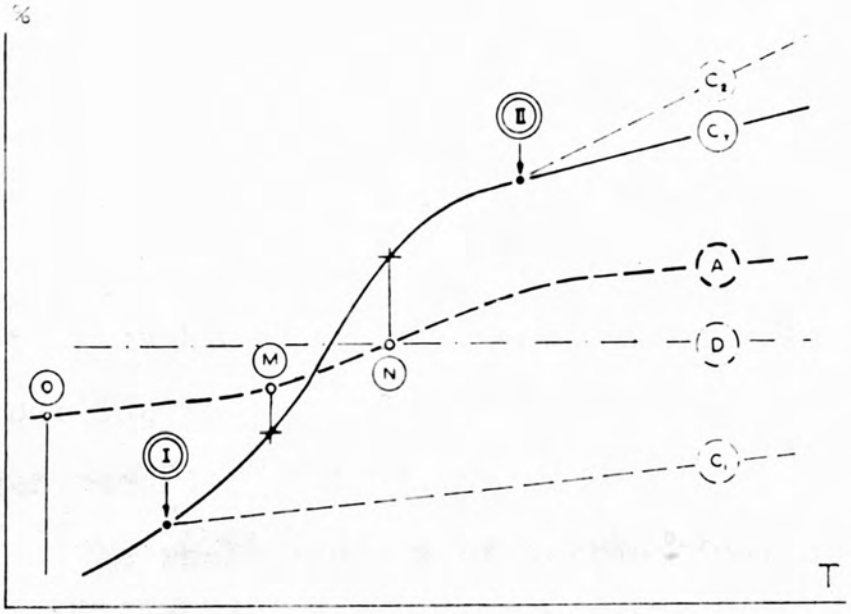
A = the curve for auxin content of the root of Lens.

O-M = the quantity of auxin is suboptimal.

M-N = the quantity of auxin increases as growth increases.

From N the concentration of auxin becomes too strong and growth falls. Above This concentration is clearly supraoptimal and consequently development is inhibited.

FIG 55



Pilet's (1950) interpretation of root growth and endogenous level of auxin is however quite contradictory. He observed in Lens that the endogenous level of auxin increases as the root increases in length. His theory is summarised in Fig.55. In the curve A (curve for auxin content of root), O-M region has low auxin, from M-N auxin increases and growth also increases and from N the concentration is supraoptimal and consequently the growth has stopped. The experimental results of this investigation with broad bean roots does not support this theory. In old roots where growth has stopped, one should find very high inhibitory concentration of growth substances according to Pilet's hypothesis, but very low amounts have been recorded instead.

The whole concept of supraoptimal or suboptimal concentration of endogenous growth substance is related to the effects of added IAA on the growth of tissue. But if it is found that IAA is not one of the native auxins of roots, the whole idea has to be altered. The difficulties of explaining root growth phenomena on the basis of supraoptimal endogenous concentration of auxin has been pointed out by Audus and Das (1955).

The disappearance of growth substances in older roots again points out directly to some inactivation process. This

inactivation, however, cannot be related to adoptive oxidation theory of Galston and Dalberg (1954). Because the results clearly show that as age increases all the growth substances fall in concentration, and only IAA (or the substance occurring at IAA zone) is not involved in the process. It may, however, be possible that a mechanism of transformation which would convert α and x into IAA ($\alpha \rightarrow$ IAA and x $\rightarrow$ IAA) exists in the root tissue. Under that condition, lowering of concentration of all these substances would be possible. But the adaptive oxidation theory cannot be applied to physiological problems related to root growth so long as there are doubts about the occurrence of IAA in roots.

In relation to ageing, what is wanted really is the picture of auxin changes as the cell ages. But the results presented here do not directly throw any light on that problem, but it shows the balance of auxins of the whole root at different ages of the plant.

But the problemswas investigated in some detail where acid auxins of tap root tips (0.5 cm. from the apex) and the rest of the main stamps and lateral roots (whole of lateral root) were chromatographically analysed at different ages of the plant (broad bean).

It was observed that in young root tips large amount of

IAA could be found which gradually fell with age and at a certain stage no trace of IAA could be observed in histograms (Fig.38.a. and Fig.40.a.). It appears that root tips lose their power to produce IAA with age. Accelerator α, however, showed an increase in early stages of growth up to 18 days, but a drastic fall in concentration followed immediately (Fig.42.a.). Unlike these two substances, accelerator x in tips remained more or less constant at a much higher level (6-10 γgm/hgm. or more; Table 6, Appendix) of concentration at all stages of growth. At the stage when the root growth has completely stopped, the high level of accelerator x signifies that it is either inhibitory to root growth or unconnected with the elongation phenomenon. But the results of root assays presented in this investigation do not throw any light on this problem. Consistent inhibition (due to inhibitor β) was recorded at the region where the oat mesocotyls show promotion of growth (Fig.27 and Fig.29). The question which naturally follows is whether substance x and inhibitor β are identical substance. That is, however, a possibility but no definite statement can be made in this relation.

In the older part of the root (i.e. root stump), the endogenous auxins present a different picture. IAA increased in concentration up to about 18-20 days and then fell to a low

value (Fig.40.b.). Accelerator α maintained a low steady concentration and after 20 days it completely disappeared (Fig.42.a.). In the stump, accelerator x shows an initial increase followed by a decrease after 18 days (Fig.42.d.). The initial increase in concentration of IAA and x in the stump must be related to emergence of new meristamatic tissue (i.e. laterals) within the main axis. It has again been observed that weight of laterals per plant does not show any increase after the 15th day (Fig.25.a.). After the emergence of the laterals, the auxins in the stump show abrupt fall in concentration.

Problems related to lateral initiation have been investigated in detail by Torrey (1950, 1952, 1956). Cell division in the pericycle leading to lateral formation has been found to be dependent on auxins, vitamins and some micro-nutrients. Normal formation of laterals has been thought to be regulated by a balance of auxin and other factors. The fact that the auxin is definitely one factor controlling root branching was shown by Thimann (1936). He also found that decapitation produced more laterals. It was earlier postulated (Zimmerman and Hitchcock, 1935) that a substance is synthesised by the primary root tip which prevents lateral branching.

From the picture of endogenous IAA of root tips (Fig.40.a.) as observed in the present investigation, it would appear that at early stages of growth high concentration of this substance inhibits lateral formation, but later (6-10 days) when the concentration falls to a relatively lower value, laterals start to appear on the stump. But the results do not explain how this inhibition is brought about.

When the lateral auxins were investigated, it was observed that IAA decreased in concentration with the age of the seedlings (Fig.41), but accelerator α and x remained more or less constant at low concentrations (Fig.42.b. and c.).

In general, the highest concentrations of growth substances were observed in the main root tip, the next highest were in the stump and in the lateral the least amount of auxins was contained. These relationships are clearly illustrated in histograms presented in Figs.31-39.

The low auxin concentration of laterals is of considerable physiological importance. It was observed again, from decapitation (of main root tip - decapitated 0.5 cm. from the tip) experiments, that in absence of primary root tip, the lateral tips synthesise more auxins. The fact that in absence of tap root tips more laterals are formed and they show more growth has been observed before by many workers and also shown

in this investigation. But it was interesting to note that growth of laterals is directly connected with its ability to produce more auxin. From repeated analysis of lateral tips of both control (tap root tips not removed) and decapitated plants of various ages (9, 16 and 20 days' old plants), it was possible to demonstrate that presence of main root tip inhibits formation of auxin in lateral tips. The basic fact that more auxin genesis in laterals accompanies more growth (Table 8) of the tissue has important bearing on hormone regulated root growth.

Certain interesting observations have been made in relation to geotropic phenomenon in roots. From chromatographic investigations of root apex material (of broad beans) from the control and the geotropically stimulated roots it was observed that such stimulation brings about a large synthesis of a substance which generally occurs at the IAA zone of the chromatogram. In order to study the nature of the synthesis, acid auxins of the root tips of control plants and plants subjected to geotropic stimulation for different lengths of time (15, 30, 35, 40 and 60 mins.), were chromatographically analysed. It was observed that the synthesis of the substance reaches its maximum at 40 mins. and then the concentration comes down to a lower value (Fig.53) at

60 mins. In pea root section test (Fig.52) it acted as an inhibitor.

The Cholodny-Went theory of geotropism has for a long time been accepted almost as a basic principle by many plant physiologists. The assumption that in roots the concentration of internal auxins is supraoptimal has not yet been accepted unanimously (Audus and Das, 1955). The proposed mechanism (i.e. redistribution theory of Cholodny-Went) depends on there being, in all positively reacting roots, an optimal or supraoptimal quantity of auxin, such that its deflection to the lower side of the root causes increased inhibition there and a release of inhibition on the upper surface.

It was quite clear from the work of Audus and Brownbridge (1957) that geotropic responses of roots cannot be explained in terms of auxin redistribution. In the present investigation, acid auxins of upper and lower halves of geotropically stimulated (60 mins.) roots were chromatographically analysed. But the results (Table 11, Appendix; also Fig.54, a, b, c and d.) do not support Cholodny-Went's theory. Slightly more auxin was observed in the upper half instead. Hawker (1932), however, provided a positive evidence for redistribution theories, following gelatin

diffusion method. In any case, the amount of error in this type of experiment is considerable. Ching and Fang (1958) observed no difference in the distribution of IAA-1-C^{14}, in the geotropically stimulated tissues (pea roots, lima bean roots and corn roots), and they recorded that differences between fresh weight of upper and lower halves were always less than 10% of their total weight.

However, from the basis of data presented in this investigation it would appear that geotropic stimulation has direct effect on the synthesis of a growth substance in the extending region of the root. Audus and Brownbridge (1957) suggested a de novo production of an inhibitor in the extending cells of the lower side of the root. Bennet-Clark et al. (1959) observed that, at the period of curvature, the rate of extension growth was markedly reduced (as did Audus and Brownbridge), and in Part I of their paper they supported and confirmed the view of previous authors (Audus and Brownbridge). But in Part II of their investigation (R. Esnault) they could not demonstrate any specific inhibitor that might be formed by sudden deflection of roots through a large angle. From starch column chromatography of root tips of Vicia faba, they further deduced that the "apparent IAA" of the tissue is almost certainly not IAA. They tended to

believe that release of material from vacuole to cytoplasm is a more probable mechanism in geotropic reaction, rather than a de novo production of growth substances. However, the results presented in this investigation directly point out that geotropic responses in roots are intimately associated with synthesis of an inhibitor (of root growth). But the substance that is produced in increased quantity by geo-stimulation could also be found in unstimulated root tip material, as also in other parts of the root. In that sense, this synthesis cannot be termed as a de novo production.

The possibility of a release of auxin from a bound state has also been investigated. If the increased production of free auxin in stimulated tips (Fig.52.c. and d.) was due to a release from the bound state, one would expect to find less bound IAA (or the substance occurring in the IAA region of the chromatogram) in stimulated tips than that in the control. But the experimental results show that the amount of bound IAA in stimulated tips is slightly more than that in the control (0.0073 µgm/µgm IAA eq. in control root tips and 0.013 µgm/µgm. IAA eq. in the geo-stimulated tips. Fig.52, e. and f.). On the basis of these observations it will be logical to conclude that the production of the hormone due to geotropic stimulation is in no way connected with the release of bound auxins.

Van Overbeek et al. (1945) observed similar production of auxin in the meristamatic node of horizontally placed stems of sugar cane. Although production of an auxin in root tips has been confirmed in this investigation, no definite conclusion can be derived as to whether or not this burst of synthesis is localised to the lower side of the elongating cells (Audus and Brownbridge, 1957). Experiments on the analysis of growth substances, however, indicate that the upper half has more auxin than the lower half. The chemical nature of the substance that is produced by geo-stimulation has not been investigated. From the Rf. value alone, it is tempting to believe that the substance is IAA. But investigations of Bennet-Clark et al. (1959) on Vicia faba root apex material suggest that IAA is present either in very low concentration or is absent in the tissue, but that the other material with Rf. near that of IAA is present in large quantity. If the substance that occurs at the IAA region of the chromatograms in the present investigation is identified as IAA, experimental evidences indicate that the possible precursor will be a TTP-like compound. Moreover, it was observed that TNH_2 does not produce IAA in broad bean root tissue.

The synthesis of this substance due to geotropic stimulus

cannot, however, be explained in terms of simple enzymatic reactions. All different possibilities need careful examination. The steady build up of concentration up to 40 mins. can be related to a synthesis from TTP-like precursor. It is again possible that a process of interconversion of the other two growth factors (accelerator α and x) into IAA (or the substance occurring at the IAA zone of the chromatogram) is working in conjunction with the process of synthesis. There are indications in the results (Figs. 47-50) that such conversion possibly takes place.

The rapid fall in concentration of the substance in the second part of the graph (Fig.53), after 40 mins. geo-stimulation, is again interesting. If the substance is found to be IAA, it can be logically explained on the basis of adaptive oxidation theory (Galston and Dalberg, 1954). But other possibilities, such the effects of limiting substrate, a reversible feed-back system, or effects of limiting concentration of the product, should also be considered. However, on the basis of the data presented here, it is difficult to predict the exact enzymatic reactions that are involved in this process of synthesis. Again, it is interesting to note that, after 60 mins. of stimulation, the concentration of the substance has come down to a relatively lower value, after

reaching the optimum production at 40 mins. But up to this period no visible curvature of the root was observed. It suggests yet another possibility, that the process of synthesis that is observed in the present investigation leads to some other reaction or reactions which are responsible for root bending.

Whichever the possibility is, the actual receptor of geo-stimulation which triggers off this enzymatic reaction still remains unknown. It would be logical to postulate that in cells a strict metabolic equilibrium is maintained and under geotropic stimulation the harmony of events at the cellular level is disturbed. Enzymatic reactions are triggered off in consequence of this disturbance. Under such conditions one should expect to find certain shifting of respiration rate. But Dr. Grobbelaar's investigations (reported by Bennet-Clark et al. 1959) on bean root apices do not furnish any positive evidence in this connection.

The physiological role of growth substances that are found to occur in the water soluble fraction presents another enigma in relation to root growth. Their break-down and spontaneous interconversion has been studied and the results are similar to those observed by Audus and Gunning (1958) in the water soluble fraction of pea root extracts. The previous

authors suggested that these substances are possibly not active in themselves, but they give rise to growth-active material in tissue segments used for bioassay. The active material that is formed could be IAA. The results of this investigation show that these auxin precursors produce promotors in shoot tissue (i.e. in oat coleoptile and wheat coleoptile segments and in oat mesocotyl segments) and inhibitors in roots (pea root segments). This holds good for all four substances separated on chromatograms. But how these inhibitors participate in the hormone control of root growth is difficult to predict.

In summing up, it can be said that in chromatographic investigation of growth substances different pictures of endogenous auxins can be obtained by following different methods of extraction, purification and bioassay. The experimental results show that endogenous acid auxins gradually fall in concentration as the seedling increases in age. In the main root tip, accelerator ∝ and IAA fall in concentration in old roots but substance x remains constant at all ages. In the root-stump, the level of endogenous auxin is possibly regulated by the initiation of lateral meristems. Again, the synthesis of auxins in lateral tips is inhibited by the

presence of the tap root tip. More growth accompanied by increased synthesis of auxin in lateral tips was observed when the tap root tips are absent. The role of water soluble auxins in root growth remains undetermined, but they ~~give~~ ~~rise~~ act ~~to~~ as an inhibitors in the root tissue. But the direct effect of geotropic stimulation has been found to be related to a synthesis of acid growth substance that generally occurs at the IAA region of chromatograms.

SUMMARY

1. In the preliminary experiments growth conditions of assay materials have been studied in relation to bioassay of growth substances from paper chromatograms.

2. It was observed in relation to the chromatography of synthetic IAA that variability in the estimated amounts of the substance arises mainly from biological variations of the segments used for bioassay, and little is due to losses in the chromatographic processes.

3. Endogenous auxins in roots have been studied using paper chromatographic methods. Oat and wheat coleoptile segment assay, oat mesocotyl segment assay and pea root segment assay were used to assay the growth active compounds from paper chromatograms. To avoid possible implications due to alkaline condition, the neutral running solvent (isobutanol : methanol : water :: 80 : 5 : 15) has been used throughout the investigation. Occasionally 70% ethanol and distilled water has also been used as the solvents.

4. In the acetonitrile purification method (developed by Nitsch) of ethanol extracts of pea roots the following growth

substances have been detected following pea root segment test: (a) Promotor, Rf. 0-0.1 (possibly accelerator α of Bennet-Clark); (b) Inhibitor, Rf. 0.1-0.3 (possibly IAA); (c) Inhibitor, Rf. 0.35-0.5 (possibly inhibitor β); (d) Inhibitor, Rf. 0.6-0.7; (e) Inhibitor, Rf. 0.8-1.0. There was also indication of the occurrence of a promotor at Rf. 0.85-0.9. Mutual interference due to close position of spots was observed.

5. Following oat mesocotyl and coleoptile segment test, four acetonitrile soluble promotors were detected in the ethanol extract of broad bean roots. They were Rf. 0-0.25 (possibly accelerator α), Rf. 0.4-0.6 (possibly IAA), Rf. 0.65-0.8 and Rf. 0.85-1.0. When similar extracts were examined following fractionation method the following growth substances were detected in different fractions:-

Acid fraction. (a) Rf. 0-0.1 (accelerator α); (b) Rf. 0.45-0.55 (possibly IAA); (c) Rf. 0.65-0.85 (inhibitor β); (d) Rf. 0.8-1.0 (accelerator x).

Neutral fraction. Rf. 0-0.1 (in water solvent).

Water soluble and ether insoluble fraction. Rfs. 0-0.1, 0.4-0.5, 0.55-0.65 and 0.8-1.0.

Growth activities of these substances in different assay methods have been studied. Two dimension chromatography of acid and water soluble auxins was attempted to identify them.

6. Water-chromatographic purification of pea root extracts indicated three inhibitors in pea root assay.

7. That different patterns of endogenous auxins could be obtained following different purification and assay methods has been established.

8. The growth substances in the water soluble and ether insoluble fraction of broad bean root extracts show spontaneous interconversion in the neutral solvent (Isobutanol : Methanol : water) used in this investigation. No such interconversion has been observed with auxins occurring in the acid fraction of the extract.

9. It was found from activity curves of acid auxins of broad bean roots that the growth effects of these substances take place within a narrow range of concentration.

10. The fate of certain synthetic growth substances and precursors within the root tissue has been investigated.

11. From the chromatographic study of the acid auxins of broad bean roots of different ages it was observed that the growth substances fall in concentration as seedlings increase in age. Initial increase in total acid auxins and IAA could possibly be related to development of lateral meristems.

12. Acid auxins from tap root tips, stump and laterals (of broad bean roots) have been chromatographically analysed and their concentration changes in different ages of plants has been studied in relation to root growth.

13. It was observed that the synthesis of acid auxins in lateral tips is inhibited by the presence of tap root tips. More growth, accompanied by increased synthesis of auxins in lateral tips, was observed when tap root tips were absent.

14. From chromatographic investigations of root apex material (of broad beans) from control and geotropically stimulated roots, it was observed that such stimulation brings about a synthesis of an acid auxin which generally occurs at the IAA zone of chromatograms. It inhibited the growth of pea root sections and promoted the growth of oat mesocotyl sections.

15. The nature of the synthesis has been studied by chromatographic analysis of acid auxins from the root tips of the control plants and the plants subjected to geo-stimulation for different lengths of time. Significance of observations has been discussed.

16. The synthesis of auxin in root tips, due to geo-stimulation, was found not to be related to the release of free auxins from the bound state.

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APPENDIX

TABLE 1.

Relation of growth of oat mescotyl sections to the concentration of IAA. Section growth expressed as % of controls. (Data of calibration curve).

Concentration of IAA mg/l.	1st Set	2nd Set	3rd Set	4th Set	5th Set	6th Set	7th Set	8th Set	9th Set	10th Set	Mean	S.D.
10^{2}	170.82	172.38	165.48	171.95	168.55	162.12	170.11	165.37	173.12	162.77	168.37	4.1
10^{1}	198.35	197.95	170.96	196.34	199.12	208.27	190.15	205.45	192.38	200.86	195.98	9.5
10^{0}	193.83	192.84	154.52	156.72	185.13	191.55	185.55	193.70	180.19	189.19	182.32	15.5
10^{-1}	179.46	191.56	151.78	165.85	178.35	171.34	155.55	178.65	166.39	180.12	171.91	12.4
10^{-2}	135.31	157.03	124.38	132.32	158.35	136.55	145.78	138.11	149.38	147.58	136.88	12.6
10^{-3}	112.73	144.25	107.95	114.62	120.76	125.45	120.67	130.12	130.19	120.12	122.63	14.2
10^{-4}	119.91	123.79	102.47	110.67	104.55	109.32	106.71	120.21	110.28	106.55	111.45	7.3
10^{-5}	104.51	116.11	118.90	104.88	103.12	101.11	100.11	118.67	-	-	108.43	7.5
S.D. of controls.	1.5	1.6	1.7	2.6	1.8	1.8	2.2	1.7	2.7	1.7	-	-
Control growth as % of original length.	30.15	31.13	29.06	26.11	23.17	28.55	30.02	24.68	32.64	28.68	-	-

TABLE 2.

Relation of growth of pea root sections to the concentration of IAA. Section growth expressed as % of controls. (Data of calibration curve).

Molar concentration of IAA.	1st Set	2nd Set	3rd Set	4th Set	5th Set	6th Set	7th Set	8th Set	Mean	S.D.
10^{-3}	28.5	21.5	24.3	17.6	16.6	10.2	16.9	18.8	19.30	5.5
10^{-4}	-	35.5	-	-	-	40.5	32.4	30.5	34.73	4.4
10^{-5}	42.1	55.5	59.2	45.4	54.1	50.3	48.8	44.5	49.99	5.6
10^{-6}	-	70.2	-	-	-	65.5	61.1	61.3	64.55	4.3
10^{-7}	62.1	90.2	78.9	83.0	66.6	90.4	72.6	80.1	67.98	14.9
10^{-8}	-	95.5	-	-	-	92.5	94.4	90.1	93.13	2.5
10^{-9}	92.8	97.8	85.6	95.7	100.0	90.6	92.3	92.1	93.36	4.1
10^{-10}	-	96.5	-	-	-	95.8	97.1	95.1	96.13	0.89
10^{-11}	107.1	105.6	101.9	103.1	108.3	102.7	110.1	109.9	106.09	3.3
S.D. of controls.	5.3	8.4	5.9	4.5	6.6	5.5	4.7	5.8	6.5	
Control growth as % of original length	78.4	82.5	80.2	108.4	90.5	70.5	100.2	92.5	91.6	

TABLE 3

Ferric chloride / Perchloric acid reagent (8 ml.) was added to 2×10^{-5} g/ml. IAA solution (2 ml.) and the resulting colour was studied at different wave lengths to determine the zone of maximum absorbency after 30 mins. of colour development at room temperature. Unicam spectrophotometer was used in the experiment.

Wave length	% transmission
450	94
460	86
480	72.5
490	65.0
500	61.0
510	58.0
520	51.0
525	50.0
527	49.9
530	49.0
535	50.0
540	53.0
550	61.9
560	73.0
570	83.0
580	89.0
590	92.0

TABLE 4.

Acetonitile soluble growth substances in Pea roots. Pea root section test.

F value	Chromatogram section no.	Growth activity* in Chromatogram A. 27 gms. 3 days old seedling.	Growth activity* in Chromatogram B. 29 gms. 3 days old seedling.	Growth activity* in Chromatogram C. 29 gms. 3 days old seedling.	Growth activity* in Chromatogram D. 30 gms 3 days old seedling.	Growth activity* in Chromatogram E. 30 gms 3 days old seedling.
-	1	112.2	122.6	87.7	120.1	120.1
0.1	2	108.1	97.4	104.5	114.4	108.5
-	3	107.4	75.5	83.2	94.4	105.4
0.2	4	103.4	87.7	76.8	82.2	70.6
-	5	87.8	78.1	89.7	76.8	74.4
0.3	6	75.7	89.0	72.3	88.3	100.3
-	7	101.4	116.8	103.9	104.4	94.5
0.4	8	87.8	72.3	109.0	98.5	76.4
-	9	81.8	71.0	96.1	80.2	65.8
0.5	10	67.6	96.1	71.0	84.6	90.3
-	11	105.4	87.7	85.8	68.8	116.2
0.6	12	70.2	105.2	63.9	110.2	86.5
-	13	98.0	80.6	128.4	91.6	72.4
0.7	14	67.6	107.1	94.8	86.5	90.4
-	15	64.2	106.6	90.3	95.4	102.3
0.8	16	100.0	72.3	111.0	104.6	112.4
-	17	81.1	78.7	83.9	94.6	94.4
0.9	18	61.5	113.2	116.1	97.6	90.4
-	19	85.8	92.3	65.8	80.2	80.6
1.0	20	91.2	116.7	68.4	86.4	76.4
Fiducial limits.		110.5 89.5	109.2 90.8	109.2 90.8	111.5 88.5	115.5 88.5

* (as % control)

TABLE 5

Auxin content (acid) of Broad bean roots in relation to the age of the seedling. Quantities expressed in IAA equivalents.

Experiment no.	Figure no. in text.	Tissue extracted.	Age of seedlings in days	Fraction	Biossay method	Yield of IAA. µgm/h.gm.	Total acid auxin µgm/h.gm.	Promotion, ↑ inhibition, ↓. Growth active regions on chromatograms are indicated as straight lines. Quantities in µgm/h.gm. .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0 R_F
44	14a	40 gms	3	Acid	Oat mesocotyl	0.72	0.931	↑0.18 ↑.027 ↓ ↑.0039
47	14b	16.5 gms	"	"	"	0.13	0.446	↑0.12 ↑.006 ↓ 0.19↑
74	14c	30 gms	"	"	"	0.64	0.807	↑ .066 ↑0.013 0.004↑
48	16a	40 gms	9	"	"	2.9	2.949	↑0.04 ↓ ↑ 0.009
54	16b	30 gms	"	"	"	0.455	0.50	↑0.025 ↑.02
75	16c	40 gms	"	"	"	1.91	2.01	↑0.05 ↑0.061
49	17a	35 gms	12	"	"	1.00	1.3	↑0.29 ↑0.01
55	17b	30 gms	"	"	"	2.01	2.02	? ↑ 0.01
55A	17c	40 gms	"	"	"	1.64	1.96	↑0.3 ↑0.015
50	18a	40 gms	15	"	"	0.047	1.596	↑ 0.049 ↑1.5
30	18b	34 gms	"	"	"	0.02	0.0504	↑ 0.0014 ↑.029
76	18c	40 gms	"	"	"	0.072	0.145	↑0.061 ↑ 0.012
51	18a	40 gms	18	"	"	0.006	0.105	↑ 0.024 ↓ ↑0.075
56	19b	40 gms	"	"	"	0.135	0.136	? ? ↑0.001
76A	19c	30 gms	"	"	"	0.083	0.129	↑ 0.026 ↑ 0.02
53	15a	40 gms	4	"	"	0.024	0.237	↑ 0.027 ↑ 0.18
57	20a	30 gms	22	"	"	0.025	0.027	↑ 0.001 ↑ 0.001
77	20b	30 gms	"	"	"	0.0157	0.0194	↑0.003 ↑0.0087
52	21a	30 gms	25	"	"	0.007	0.007	? ↓ ?
78	21b	40 gms	"	"	"	0.01	0.019	↑0.009 ?
79	21c	40 gms	"	"	"	0.003	0.0085	↑ 0.003 ↓ ↑ 0.0025

TABLE 6

Neutral auxins in Broad bean roots.

Experiment no.	Tissue extracted.	Age of seedlings in days	Fraction	Solvent	Growth active regions are indicated as straight lines promotion ↑ inhibition. ↓ . .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0 R_F
44	40 gms	3	Neutral	Isolbutanol: Methanol: H_2O	
47	16.5 gms	"	"	"	
54	30 gms	9	"	H_2O	
49	35 gms	12	"	Isolbutanol: Methanol: H_2O	
55	40 gms	"	"	H_2O	
50	40 gms	15	"	Isobutanol: Methanol: H_2O	
51	40 gms	18	"	"	
56	40 gms	"	"	H_2O	
52	30 gms	25	"	Isobutanol: Methanol: H_2O	
57	30 gms	22	"	"	
53	40 gms	4	"	H_2O	
30	34 gms	15	"	Isobutanol: Methanol: H_2O	

TABLE 7

Plants grown black perspex holder - shoot in light - root in dark.
Standard deviations (±) are noted against readings.

Age of seedlings	Root lenght 5-6 c m at the end of 72 hrs. Group A.		Root length 3-5 c m at the end of 72 hrs. Group B.		Root length 2-3 c m at the end of 72 hrs. Group C.		Root length 1-2 c m at the end of 72 hrs. Group D.	
	Shoot length c m.	Root length c m.	Shoot length c m.	Root length c m.	Shoot length c m.	Root length c m.	Shoot length c m.	Root length c m.
72	0.76	5.14	0.45	4.09	0.14	2.29	0.05	1.28
96	2.52 ± .31	6.83 ± .37	1.48 ±0.21	7.43 ±0.41	0.87 ±0.33	3.98 ±0.46	0.34 ±0.31	2.15 ±0.37
120	3.33 ±0.65	10.15 ±0.88	2.16 ±0.57	9.06 ±0.71	1.30 ±0.39	6.49 ±0.46	0.68 ±0.22	2.85 ±0.44
144	3.75 ±0.53	12.25 ±1.42	3.14 ±0.48	10.84 ±1.05	2.26 ±0.54	7.40 ±0.35	1.41 ± .51	4.08 ±0.48
168	3.54 ±0.33	12.52 ±1.05	3.48 ±0.32	12.04 ±1.29	2.70 ±0.40	8.61 ±0.48	2.24 ±0.32	4.09 ±0.35

TABLE 8

Distribution of acid auxin in Broad bean roots.

Experiment no.	Figure no. in text.	Zone of root.	Tissue extracted.	Age of seedlings in days.	Fraction	Bioassay method	Yield of IAA. µgm/kg.	Total acid auxin accelerators µgm/kg.
62 a.	31a	Root tips from 132 pl.	3 gms	4	acid	Mesocotyl	6.24	–
62 b.	31b	Older part " "	31 gms	"			0.023	0.1
63 a.		Root tips from 181 pl.	1.5 "	10	"	"	3.13	14.682
63 b.		Root stump " " "	25.7"	"	"	"	0.411	0.541
63c.		Root laterals " "	30 "	"	"	"	0.16	0.201
61 a.	36a	Root tips from 59 pl.	1.5 "	18	"	"	1.16	16.48
61 b.	36b	Root stump " " "	31 "	"	"	"	0.527	0.940
61 c.	36c	Root laterals 25 "	35 "	"	"	"	.023	0.051
64 a.	38a	Root tips from	0.7 "	34	"	"		–
64 b.	38b	Root stump	20 "	"	"	"	.079	0.175
64 c.	38c	Root laterals	31.0"	"	"	"	.0016	0.039
68 a.	39a	Root tips	1.6 "	3	"	Pea root	13.4	–
68 b.	39b	Root laterals	30 "	18	"	" "	.0015	.0015
68 c.	39c	" "	35 "	34	"	" "	.0025	.0025
80 a.	34a	Root tips	1.5 "	15	"	Oat meso-cotyl	2.7	14.9
80 b.	34b	Root stump	30 "	"	"	" "	0.34	0.55
80 c.	34c	Root laterals	30 "	"	"	" "	0.0093	0.0411
73	32	Root tips	1.2 "	4	"	Pea root	14.8	15.43
63Aa.	33a	Root tips	1.5	10	"	Oat meso-	3.2	10.93
63Ab.	33b	Root stump	35	"	"	cotyl "	0.11	0.179
63Ac.	33c	Root laterals	32	"	"	" "	0.04	0.055
80Aa.	37a	Root tips	1.5	25	"	" "	0.0025	2.203
80Ab.	37b	Root stumps	35	"	"	" "	0.054	0.119
80Ac.	37c	Root laterals	35	"	"	" "	0.0057	0.0614
64Aa.	35a	Root tips	1.1	16	"	" "	1.3	8·26
64Ab.	35b	Root stump	40	"	"	" "	0.396	0·728
64Ac.	35c	Root laterals	40	"	"	" "	.019	0·3007
61Aa.		Root tips	1.2	20	"	" "	1.4	8·12
61Ab.		Root stumps	35	"	"	" "	0.46	0·68
61Ac.		Root laterals	35	"	"	" "	0.012	0·034

Promotion, ↑ inhibition, ↓. Growth active regions on chromatograms are indicated as straight lines. Quantities in µgm/h.gm.

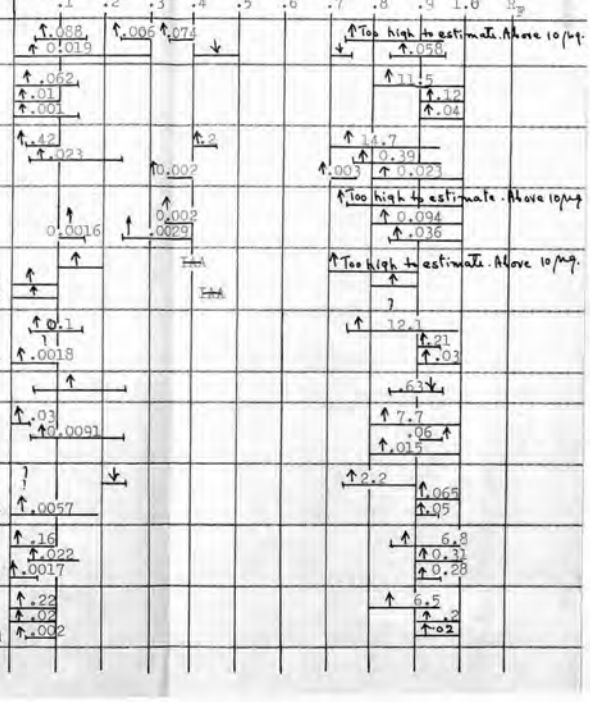

TABLE 9

Effects of decapitation on root auxin (acid)

Experiment no.	Figure no. in text.	Treatment and tissue	Tissue extracted.	Age of seedlings in days	Fraction	IAA µgm/hg.	Total acid auxin µgm/hg.	Promotion ↑ inhibition ↓ Growth active regions on chromatograms are indicated as straight lines. Quantities in µgm/hg.
67 a	43a	Control plants.	30 gms	9	acid	0.43	0.91	↑.083 ; ↑0.4
b	43c	Decapitated plants. (Decapitation 3rd day of sowing.	30 "	"	"	1.51	3.435	↑.001 ; ↑.044 ; ~~IAA~~ ; ↑1.88
c	43b	Control plants.	30 "	"	neutral	-	-	no activity on the chromatogram
d	43d	Decapitated plants. (Decapitation 3rd day of sowing. Oat mesocotyl assay	30 "	"	"	-	-	↑
72 a	44a	Control plants. (pea root assay)	30 "	"	acid	0.42	0.446	.006↓ ; ↓.02
b	44b	Decapitated plants. (pea root assay). Decap. 3rd day of sowing	30 "	"	"	.95	0.983	.002↓ ; ↓.03
70 a		Root stumps of control plant (with tap root tips).	29 "	"	"	0.017	0.07	.001↓↑ ; ↓ ; ↑.05 ; ↑.002
b		Root stumps of decap. plant.	20 "	"	"	0.21	0.208	↓ ; ↑0.008
c		Lateral root tips of cont. pl.	1 "	"	"	0.26	0.42	? ; ↑.06 ; ↑.1
d		" " " " decp. pl. Oat mesocotyl assay	1 "	"	"	2.5	3.33	.29↑ ; ↑.54
70Aa	45a	Lateral root tips of control plants - 300 tips - .5 cm (Collected from 8 plants)	1 "	16	"	0	3.65	↑.09 ; ↑3.56
b	45b	Lateral root tips from decapitated plants - 310 tips - .5 cm (Decap. on 5th day of sowing).	1 "	"	"	1.0	-	↑.2 ; ↑More than 10 µgm.
c	45c	Tap root tips from 72 plants	0.8 "	"	"	0.37	-	↑.025 ; ↑More than 10 µgm.
d	45d	76 stumps from decapitated roots.	28 "	"	"	0.48	2.57	1.4↑ ; ↑0.69
e	45e	50 stumps from control plants. (Tips assayed separately) Oat mesocotyl assay	32.5"	"	"	1.2	4.74	0.04↑ ; ↑3.5
70Ba	46a	Lateral root tips of control plants - 312 tips - .5 cm.	1 "	20	"	-	1.62	0.12↑ ; ↑1.5
b	46b	Lateral root tips of decapitated plants - 310 tips - .5 cm. Oat mesocotyl assay	1 "	"	"	2.01	-	.4↑ ; ↑More than 10 µgm.

TABLE 10

Effect of geotropic stimulation - acid fr. chromatographed.

Experiment no.	Figure no. in text.	Treatment and tissue	Presentation time for geo. stiml.	Tissue extracted.	Age of seedling in days	Bioassay method	IAA µgm/gm.	Promotion ↑ inhibition ↓ Growth active regions on chromatograms are indicated as straight lines. Quantities in µgm/gm wt. .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0 R_F
73 a	52a	5 m m. root tips from Control plants. 108 r.tips.	-	1.2 gms.	3	Pea root	.22	↑ 0.0065↓
b	52b	5 m m. root tips from geotropically stimulated roots. 106 r.tips	1 hour	1.1 gms	3	Pea root	142.65	↓ ↑ ↓ 0.005↓
73Aa	51a	5 m m. root tips from Control plants. 110 r. tips	-	1.0 gms	3	Oat mesocotyl.	1.03	↑.004 ↑10.23
b	51b	5 m m. root tips from geotropically stimulated plants. 115 r. tips.	1 hour	1.0 gms	3	Oat mesocotyl.	28.33	↑.0002 .0076↑ ↓

TABLE 11

Experiment no.	Figure no. in text.	Treatment and tissue	Tissue extracted	Age of seedlings in days	Fraction	IAA µgm	Total acid auxin µgm.	Promotion ↑ inhibition ↓. Growth active regions on chromatograms are indicated as straight lines. Quantities in µgms present in the tissue. .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0 R_F
73B	54a	Broad bean roots - (3 days old) were stimulated geotropically for one hour.						
		(a) upper half - 101 tips - 0.5 c m.	0.5 gms	3	Acid	0.078	0.0803	↑.002 ↑.0003
		(b) lower half - 101 tips - 0.5 c m.	0.48 gms	3	Acid	0.011	0.0123	↑.0003 ↑.001
73C	54c	Broad bean roots - (3 days old) were stimulated geotropically for one hour. (Plants that were used in 73B were used here).						
		(a) upper half - 104 tips - 0.5 c m.	0.59 gms	3	Acid	0.052	.0535	↑.0006 ↑.0009
		(b) lower half - 104 tips - 0.5 c m.	0.59 gms	3	Acid	0.022	.0274	↑.0004 ↑.005

TABLE 12

Experiment no.	Figure no. in text	Treatment and tissue	Tissue extracted	Age of seedlings in days	Fraction	IAA µgm/gm wt.	Total acid auxin µgm/gm wt.	Promotion ↑ inhibition ↓. Growth active regions on chromatograms are indicated as straight lines. Quantities in µgm/gm wt. .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0 R_F
98a	52c	Free acid auxins in control root tips (112 root tips 0.5 c m from the apex).	1.3	3	Acid	0.006	-	↑ 0.005
98b	52d	Free acid auxins from geo. stimulated (for 40 mins) root tips (110 root tips - 0.5 c m from the apex)	1.1	3	Acid	1.9	1.9	↑ Too high to estimate.
99a	52e	Bound acid auxins from the same tissue as in 1.	1.3	3	Acid	0.007	0.579	↓ ↓ ↑ 0.572
99b	52f	Bound auxins from the same tissue as in 2.	1.1	3	Acid	0.013	0.747	↑ 0.734

Table 13

Oat coleoptile assay results of the primary chromatogram of the elution and rechromatography experiment of acid auxins.

Chromatogram section no.	R_F values.	Growth activity in chromatogram.
1	-	120.4
2	0.1	125.3
3	-	115.5
4	0.2	90.5
5	-	95.4
6	0.3	105.5
7	-	108.6
8	0.4	98.4
9	-	110.8
10	0.5	114.9
11	-	130.2
12	0.6	114.9
13	-	105.4
14	0.7	85.5
15	-	69.1
16	0.8	81.3
17	-	88.8
18	0.9	90.5
19	-	100.1
20	1.0	102.1

Fiducial limits of control sections - 108.1 and 91.9 (above and below the 100% level).