STUDIES OF THE GROWTH SUBSTANCES OF LEGUME NODULES USING PAPER CHROMATOGRAPHY

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Summary

Five distinct growth substances active in the Avena coleoptile straight-growth test were separated by paper chromatography of ethanol extracts of nodules of Pisum arvense L. and Ulex europaeus L. Three of the active substances were classed as promotors, two as inhibitors, of coleoptile elongation. They exhibited the following migration behaviour in isopropanol-ammonia chromatography solvent:

- Accelerator 1 (A1): acidic, $R_F$ 0·05–0·15.
- Accelerator 2 (A2): acidic, $R_F$ 0·30–0·45.
- Accelerator 3 (A3): non-acidic, $R_F$ 0·70–0·85.
- Inhibitor 1 (I1): non-acidic, $R_F$ 0·50–0·60.
- Inhibitor 2 (I2): non-acidic, $R_F$ 0·75–0·85.

$A_3$ was identified colorimetrically and by ultraviolet fluorescence as indole-acetic acid. The chemical identity of the other constituents was not determined but similarities in migration and fractionation were noted between $A_1$ and the $a$-accelerator of Bennet-Clark and Kefford (1953), and between $A_3$ and the indoleacetonitrile of Cruciferae extracts.

In terms of Avena response, $A_3$ (indoleacetic acid) was designated as the dominant auxin of Pisum nodules, being recovered in large amounts at all stages of nodule growth. $A_1$ first appeared in chromatograms of young, recently pigmented nodules, and thereafter retained a fairly uniform activity until nodule senescence. $A_2$ appeared in quantity during the second week of nodule life, but diminished to minute or undetectable amounts as nodules aged. Both inhibitors were consistent features of tissue extracts and apparently increased in concentration with nodule growth.

There was no observable qualitative or quantitative decrease in Pisum nodule growth substances as haemoglobin decomposition took place in senescent nodules.

The meristematic and bacterial portions of the Ulex nodule contained approximately similar amounts of the nodule growth substances.

All Avena-active substances of nodule extracts except $A_3$ were recorded from assays of Pisum root extracts. Extractable auxin levels were found to be very much lower in root tissues than in nodules.

I. INTRODUCTION

In the earliest accounts of symbiosis it was suggested that nodule initiation might be a direct consequence of the diffusion of active substances produced by bacterial synthesis in the infected tissues of the legume root (see Fred, Baldwin, and McCoy 1932; Wilson 1940; Thimann 1955). The present trend of thought still

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ascribes a dominant role to growth substances in nodule genesis, although the best evidence still fails to prescribe the origin and identity of chemical agents specifically responsible for mitotic stimulation in nodule development.

Interest in nodule auxins resulted largely from the researches of Thimann (1936, 1939) and Thimann, Skoog, and Byer (1942) who used the *Avena* coleoptile curvature test to demonstrate that nodules contained diffusible auxins in much greater concentration than neighbouring root tissues. Auxin production in nodules paralleled nodule growth; auxin occurred in excessive amounts only in infected tissues; apical halves of nodules yielded similar amounts of diffusible auxin to basal halves; and degenerate nodules contained little, if any, auxin. Assays of nodules conducted by Link and Eggers (1940) and Egle and Munding (1951) have also described nodule tissues as being very rich in extractable auxins.

The bacteria themselves have been considered to be responsible for auxin syntheses in the nodule. Production of indoleacetic acid by *Rhizobium* in various culture media containing tryptophan has been demonstrated by Link (1937), Chen (1938), Thimann (1939), and Georgi and Beguin (1939).

Thimann (1939) proposed that the physiology of nodule formation was pivoted on the activity of the auxins produced by the nodule bacteria. He depicted nodule genesis as a similar process to lateral root development, but where the maintenance of supraoptimal levels of growth substances prevented elongation of infected tissues into a structure resembling a root. This concept of the nodule as an arrested secondary root has been rejected by Wilson (1940), Bond (1948), and Allen and Allen (1953) who stress that the nodule is a unique type of root hypertrophy in being of cortical origin and of markedly different structure and function from a secondary root.

Application of various growth substances to roots have failed to discover stimulants of cortical proliferation, if such substances exist. Kraus (1941), Bond (1948), and Allen, Allen, and Newman (1953) have all reported negative findings in this respect, although the last-named authors produced pseudonodular outgrowths on legume roots by application of 3,5-dichlorobenzoic acid, and Bond (1948) described localized vascular differentiation in roots following application of tryptophan.

The extensive investigations by Nutman on the physiology of nodule formation in red clover and other legumes have given adequate evidence of hormone-type interactions in nodulation (see Nutman 1956). Seedling root excretions have been described as influencing the extent and precocity of nodulation in agar cultures of legumes, while characteristic internodular reactions have been demonstrated for later symbiotic development. The chemical identities of the respective active substances are apparently unknown.

The research detailed in this paper was originally designed as a quantitative analysis of auxins in the maturing nodule. Preliminary studies showed marked antagonism between auxins, inhibitors, and interfering impurities in nodule extracts which prevented use of conventional colorimetric or biological assays. Consequently the technique of paper chromatography coupled to *Avena* assay was explored as an alternative method of comparing tissue extracts. This proved satisfactory for a semi-quantitative description of nodule growth substances in aging nodule tissues.
II. Materials and Methods

(a) Extraction of Growth Substances

A standard, low-temperature ethanol extraction procedure followed by hexane-acetonitrile purification was used in all analyses described in this paper:

(i) The fresh tissue was ground with half its volume of quartz sand and covered with 10 times its weight of absolute ethanol. Extraction was allowed to proceed for 20 hr at $-15^\circ$C.

(ii) The extract was filtered, the ground tissue washed three times with small volumes of ethanol, and the combined extract then evaporated to dryness under reduced pressure (temperature kept below 50°C).

(iii) The residue from evaporation was redissolved in 25 ml acetonitrile and partitioned with five washings of 20 ml hexane (B.P. 68°C) (see Nitsch 1953). Trial chromatograms showed that the hexane removed lipoidal impurities while apparently all of the extracted growth substances were retained in the acetonitrile fraction.*

(iv) The acetonitrile layer was reduced to small volume by evaporation under reduced pressure and applied to the chromatogram as a total extract.

(v) In some experiments the purified extract from (iii) was fractionated into acidic and non-acidic portions by the method of carbonate to phosphoric acid transfer given by Bonde (1953).

(b) Chromatography of Extracts

Ascending-descending chromatography on Whatman No. 1 paper was used with isopropanol-ammonia (sp. gr. 0·880)–water (80 : 5 : 15 v/v). According to Stowe and Thimann (1954) this is the most versatile solvent, and it has been widely used by many workers in the auxin chromatography field. The solvent gave satisfactory separation of the active fractions of nodule tissues. The following chromatography procedure was used: equilibration, 3 hr; running time, 11–15 hr at 24°C; solvent-front coverage, 25–35 cm; position of indoleacetic acid (IAA) marker spot run under these conditions, $R_F$ 0·35–0·45; position of indoleacetonitrile (IAN) marker spot, $R_F$ 0·70–0·80.

Chromatograms were dried by heating for 10–15 min at 35°C. This procedure removed all traces of solvent from the paper. Chromatograms not immediately required for bioassay were stored in an atmosphere of nitrogen in the dark at $-15^\circ$C.

(c) Bioassay of Chromatograms

The biological assay method selected was the Avena coleoptile straight-growth test as developed and refined for auxin chromatography work by Bentley (1950) and Bentley and Housley (1954).

*Nitsch (1953) recommends double chromatography as an ideal method of eliminating waxes from crude wax-free or alcoholic extracts. A preliminary run, with water as solvent, is followed by elution of wax-free portions of the paper and a second chromatographic development in normal solvent. This method proved satisfactory in one series of nodule extracts but was eventually discarded as it involved 3 days of chromatography and final activities of extracts were lower than those obtained from the acetonitrile purification method.
(i) *Elution of Chromatography Segments.*—Chromatograms were examined under ultraviolet light to guide selection and sectioning. Each chromatogram was sectioned into 20 equal segments (i.e. each segment representing 0.05 of an $R_f$ unit). This 20-strip analysis was found necessary for the successful delimitation of active areas, particularly in total extracts containing a variety of adjacent growth-inhibiting and growth-promoting substances.

Segments were eluted for 3 hr in 5-ml petri capsules containing 5 ml 3 per cent. sucrose containing citrate–phosphate buffer as recommended by Nitsch (1953). A carbohydrate source was found to be essential in the *Avena* assay to promote sufficient control growth for the detection of inhibitor activity on chromatograms.

(ii) *Growth of Avena Coleoptiles.*—Svalof Victory oats were soaked for 14–16 hr in tap-water at 10°C and sown in batches of 2000 in large enamel trays (14 by 16 by 2 in. deep) containing 4 l. of wet, sterile river sand. The trays were placed in the dark in a humidity chamber maintained at 25°C. Mesocotyl growth was suppressed by giving emerging coleoptiles a 5-min exposure to daylight 24 hr before harvest. Coleoptiles were harvested 72 hr after sowing.

(iii) *Bioassay Technique.*—The complete bioassay was set up in a dark room under red light. Coleoptiles were selected from the size range showing 15–18 mm growth above the mesocotyl, and were guillotined on a special cutter which removed a 10-mm subapical portion and rejected a 3-mm tip and the basal portion of the coleoptile. Cut coleoptiles were randomly distributed among the dishes of chromatogram segments until each dish contained ten 10-mm sections. Ten capsules, with a strip of filter paper and 5 ml of 3 per cent. buffered sucrose, were included as medium control series for each bioassay.

The coleoptile sections were grown in darkness for 24 hr at 25°C. At the conclusion of the growth period, the lengths of the 10 sections in each capsule were recorded by measuring enlarged microprojector images of the coleoptile with an opisometer.

(d) *Presentation of Results*

The *Avena* activity of each chromatogram was expressed in conventional histogram form where mean coleoptile extension of consecutive chromatogram segments was represented as a percentage of the medium control growth. Significance levels were drawn on each histogram at $\pm 2$ times the standard deviation of coleoptile elongation in the 10 dishes of the medium control series. Values on the histograms outside these limits were taken as significant ($P<0.01$) evidence of the presence of *Avena*-active growth substances.

### III. Results

(a) *Qualitative Examination of the Growth Substances in Aging Nodules of the Field Pea,* *Pisum arvense* *L.*

Eight samples of primary root nodules for chromatogram series 1 were taken from soil-grown field pea plants in September 1955 in the sequence shown in Table 1. Effective nodules were selected from the top 4 in. of primary root to confine developmental effects to a similarly aged and sized nodule population.
A second series, chromatogram series 2, was designed to check some of the qualitative changes in nodule auxins noted in the 1955 series. A similar sowing of field pea was made and three age groups of nodules selected for detailed study in September 1956:

Set A: young white nodules, 6-, 3-, and 1-g samples from 2-leaf plants (equivalent to the 13-day stage, 1955 series).

Set B: young red nodules, 7-, 4-, 2-, and 1-g samples from 4-leaf plants (equivalent to the 20-day stage, 1955 series).

Set C: older red nodules, 8-, 4-, 2-, and 1-g samples from 8-leaf plants (equivalent to the 32-day stage, 1955 series).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Days after Sowing</th>
<th>Leaves per Plant (age)</th>
<th>Fresh Wt. of Nodule Sample (g)</th>
<th>% of Nodules with Haemoglobin Pigment</th>
<th>Approx. No. of Nodules in Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>2</td>
<td>8</td>
<td>48</td>
<td>8010</td>
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<tr>
<td>2</td>
<td>16</td>
<td>3</td>
<td>8</td>
<td>79</td>
<td>6650</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>4·3</td>
<td>8</td>
<td>98</td>
<td>4050</td>
</tr>
<tr>
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<td>5</td>
<td>4</td>
<td>100</td>
<td>1400</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>6·9</td>
<td>4</td>
<td>100</td>
<td>890</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>8·1</td>
<td>4</td>
<td>100</td>
<td>670</td>
</tr>
<tr>
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<td>9·4</td>
<td>4</td>
<td>98</td>
<td>600</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>10·4</td>
<td>4</td>
<td>93</td>
<td>410</td>
</tr>
</tbody>
</table>

Activity histograms of series 1 and 2 are presented in Figures 1 and 2 respectively. The following tentative conclusions may be drawn from the results of both series:

(i) Five characteristic growth substances can be detected in the Avena assay of nodule extracts. The following names and \( R_F \) values are assigned to the active areas described on the histograms and subsequently mentioned in the text:

<table>
<thead>
<tr>
<th>Growth Substance</th>
<th>( R_F ) at which Maximum Activity Occurs</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_1 )</td>
<td>0·05–0·15</td>
</tr>
<tr>
<td>( A_2 )</td>
<td>0·30–0·45</td>
</tr>
<tr>
<td>( A_3 )</td>
<td>0·70–0·85</td>
</tr>
<tr>
<td>( I_1 )</td>
<td>0·50–0·60</td>
</tr>
<tr>
<td>( I_2 )</td>
<td>0·75–0·85</td>
</tr>
</tbody>
</table>

(ii) \( A_1 \) becomes detectable on chromatograms of extracts from nodules of 3-leaf plants (i.e. just after the majority of nodules have turned red). It is recovered from all older nodule samples.
Fig. 1.—Growth substances in aging nodules of P. arvense. Avena-activity histograms of chromatograms of total alcohol extracts of samples of eight age groups of primary root nodules from soil-grown field pea plants. Details of the nodule samples are given in Table 1. (In the histograms of Figures 1–4 mean coleoptile extensions of consecutive chromatogram segments (RF scale) are represented as a percentage of coleoptile growth in pure assay medium (vertical scales). Significance levels are drawn at ±2 times the standard deviation of coleoptile elongation in the medium control series. The positions of marker spots of IAA and IAN and of the typical RF values assigned to the five nodule growth substances are included at the top of each set of histograms.)
(iii) \( A_2 \) is present in large amounts at all stages of nodule development. There is evidence of supra-optimal activity in the \( A_2 \) zone in several histograms (e.g. the 8-g samples of series 1 (Fig. 1) and 7-g sample of series 2 (Fig. 2)). In series 2 there is some evidence of a slight decrease in \( A_2 \) concentration in the aging nodule, particularly after 5 weeks of nodule growth.

![Diagram](image)

Fig. 2.—Growth substances in aging nodules of \( P. \ arvense \). \( Avena \)-activity histograms of chromatograms of total alcohol extracts of varying quantities of tissue from three age groups on nodules (chromatogram series 2, see text).
(iv) $A_3$ is absent from very young nodules but appears in quantity in the samples from 4-leaf plants. Thereafter $A_3$ activity diminishes until, at the 8-leaf stage of plant development, it is barely detectable from nodule tissues.

(v) It is difficult to provide reliable information on fluctuations in the *Avena*-inhibitory substances of the nodule. $I_1$ may be masked by overlap with high $A_2$ concentrations, and $I_2$ may be similarly antagonistic to $A_3$ activity. These antagonisms are demonstrated clearly in the series 2 chromatograms (Fig. 2). The sporadic occurrence of $I_1$ and $I_2$ throughout the samples would indicate that both are a consistent feature of tissue extracts. There is some evidence from both series that inhibitors are more concentrated in older nodules.

(vi) The three stages examined in series 2 confirm the auxin changes described in series 1 and also provide evidence of a change in relative concentration of the three accelerators. $A_3$ is the only accelerator visible in bioassay of extracts of young white nodules; young red nodules have the three accelerators present in relative proportions $A_2>A_3>A_1$; in older nodules the order of concentration changes to $A_3>A_1>A_2$.

(b) Further Characterization of Nodule Growth Substances

(i) Chemical Identification of Nodule Growth Substances.—A 250-g nodule sample from *Pisum* plants was used for this analysis. The purified extract was run as a long strip on the chromatogram. Strips of the developed chromatogram were treated with ferric–perchloric reagent* and with Ehrlich’s reagent† and gave characteristic IAA colour reactions in the 0·30–0·50 $R_F$ region. Ultraviolet illumination of untreated strips showed typical ash-coloured fluorescence in the same $R_F$ region giving additional confirmation of the presence of IAA in nodule tissues. Identifiable colorimetric and fluorescence reactions could not be obtained from other regions of the chromatograms.

(ii) Fractionation of Nodule Growth Substances into Acidic and Non-acidic Constituents.—Figures 3(a) and 3(b) detail sample activity bioassay histograms of chromatograms of acidic and non-acidic fractions of nodule extracts. Equal weights (3 g) of apical and basal portions of nodules of *Ulex* were assayed (Fig. 3(a)). There was no evidence of differences between the constituents of the two halves of the nodules. $A_1$ and $A_2$ accumulated in the acid fractions, $I_1$ and $I_2$ in the non-acidic fractions. Figure 3(b) shows a typical activity histogram following fractionation of extracts of young *Pisum* nodules. $A_1$ and $A_2$ are again designated as acidic substances and $I_1$, $I_2$, and $A_3$ are non-acidic. In both series there is evidence of a slight IAA “leak” into the non-acidic fraction.

(iii) Growth Substances in the Senescent Nodule.—Several extracts of red and green nodules of similar age were compared in both *Pisum arvense* and *Ulex europaeus*. A typical set of histograms is depicted in Figure 3(c). All the activity zones except $A_3$ are reproduced in green and red samples and there is no evidence of any extensive destruction of any of the growth substances coincident with haemoglobin destruction in early nodule senility.

*50 parts of 5 per cent. perchloric acid to 1 part 0·05M ferric chloride.
†1 per cent. p-aminobenzaldehyde in 1N hydrochloric acid.
Fig. 3.—(a₁)–(a₄) Avena-activity histograms showing typical comparisons of chromatograms of the acid and non-acid fractions of alcohol extracts of basal and apical portions of 1-year-old nodules of Ulex europaeus. (a₁) 3 g apical portions, acid fraction; (a₂) 3 g basal portions, acid fraction; (a₃) 3 g apical portions, non-acid fraction; (a₄) 3 g basal portions, non-acid fraction. (b₁), (b₂) Comparison of the Avena-activity histograms of chromatograms of the acid (b₁) and non-acid (b₂) fraction of an alcohol extract of 5 g of red nodules of P. arvense. (c₁), (c₂) Comparison of the Avena-activity histograms of chromatograms of total alcohol extracts of 4-g quantities of red (c₁) and green (c₂) nodules from 84-day-old plants of P. arvense. (d₁)–(d₄) Avena-activity histograms of chromatograms of total alcohol extracts of 40 g (d₁), 20 g (d₂), and 10 g (d₄) of Brussels sprouts tissue.
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(iv) Comparison of Nodule and Brussels Sprouts Extracts.—The activity histograms of 40-, 20-, and 10-g portions of Brussels sprouts tissue (Brassica oleracea) are included in Figure 3(d) solely for comparative purposes. Marked activity in the IAN region is noted in the same position as the A₃ activity of nodules. Activity in the A₃ region is represented in this Cruciferaceae extract in apparently more active proportions than is the A₂ (? IAA) activity zone.

![Graphs showing activity histograms and calibration curves](image)

Fig. 4.—Typical activity histograms of chromatograms of total alcohol extracts of nodule and root tissues from 54-day-old plants of P. arvense. A calibration curve of coleoptile elongation against the logarithm of IAA concentration is included for comparison of the extractable auxin levels in the two tissues (see text).

(v) Comparison of Nodule and Root Extracts in Pisum.—Growth substance activities of 10 and 40 g of Pisum roots are recorded in Figure 4. Root auxin levels are seen to be very much lower than in nodules, although all of the five zones showing Avena-activity zones, except A₃, are recorded for roots. Activity of 1-g and 4-g Pisum nodule extracts can be compared with the root extract activities by reference to a calibration curve of coleoptile extension against the logarithm of IAA concentration. It is estimated that IAA-extractable activity in roots is some 40–60 times lower than in nodule tissues of similar age (cf. similar report by Thimann, Skoog, and Byer 1942).

High concentrations of I₁ and I₂ are recorded for root tissues (cf. reports of inhibitor action in roots by Howell 1954; Torrey 1956).

IV. DISCUSSION

Chromatograms of ethanol extracts of Pisum and Ulex nodules and Pisum roots show five characteristic zones of activity in the Avena straight-growth test. Of these, three are classed as promoters (A₁, A₂, A₃) and two as inhibitors (I₁, I₂) of coleoptile elongation.

A₂ is identified chemically as indoleacetic acid (IAA) confirming previous reports of the presence of this growth substance in legume nodules by other workers. The chemical identity of the other four constituents cannot be specified. The R₉ and
fractionation behaviour of $A_1$ are similar to those of the $\alpha$-accelerator described by Bennet-Clark and Kefford (1953). This substance has been described for a variety of tissues including legume roots (see Kefford 1955). On similar evidence, $A_3$ may be associated with indoleacetonitrile (IAN), the neutral component which has been isolated from Cruciferae extracts (Jones et al. 1952). Unfortunately, owing to the small amounts in nodule tissues, it was not possible to hydrolyse $A_2$ zones on chromatograms to see if IAA was produced, as would be expected from an IAN source. Of the two inhibitors, $I_1$ has similar migration behaviour to the $\beta$-inhibitor of Bennet-Clark and Kefford (1953), yet $I_1$ is recorded as a non-acidic substance. $I_2$ cannot be identified with any "named" activity area mentioned by other authors. However, large amounts of Avena-inhibitory substance have been recorded from this high $R_f$ region from extracts of bean plumules (Phaseolus) and stolons of blackberry (Rubus) (Gunning and Pate, unpublished data.)

The qualitative analysis of growth substances in developing Pisum and Ulex nodules depicts an ordered sequence of auxin changes, but fails to provide any basis for connecting these events with the fundamental growth pattern of the nodule. The usual criticisms of this type of auxin assay apply here. It is not known whether the ethanol extraction method used here is a wholly satisfactory method of estimating diffusible auxin activity: interconversions between indole compounds are known to occur in some extraction procedures. Are the extracted substances identified as being active in monocotyledon cell elongation of definite function in the tissues of the legume nodule, or even present in the nodule in suitable condition for physiological activity?

Despite the above limitations on interpretation the results demonstrate that nodule tissues are centres of very intensive growth substance activity, particularly IAA activity. In all probability the nodule is self-contained in its auxin syntheses. As yet it is not known whether nodule auxins are metabolic products of the microsymbiont, substances released by host cells in response to bacterial irritation, or, as is more likely, represent a synergy of host and bacterial processes. In the latter case it might be held that the rhizobia were supplied with suitable precursors from host cell proteolytic activities.

The presence of similar growth substances in root and nodule tissues would suggest the presence of similar synthetic mechanisms within these organs, though it is difficult to explain why there are such large quantitative differences in the diffusible auxin contents of nodule and root. Oxidative enzymes may be absent or blocked, as suggested by the work of Wagenknecht and Burris (1950), or high levels of auxin may accumulate in nodules simply because normal oxidase systems cannot cope with the intensive and continued synthetic activities of the intracellular bacteria.

A further question is whether there is a substantial backward diffusion of nodule growth substances into parent root tissues. If there is, there may be a mechanism of root inactivation of these substances, possibly by oxidase systems or by acceptance into bound complexes. Nutman's (1936) studies give evidence of internodular inhibitions elicited by excretions from nodule meristems. It is tempting to suggest that these inhibitory effects might be connected with a backward diffusion of nodule
auxins and inhibitors. Further observations of internodular reactions will be provided in a further paper on the effect of delayed inoculation on legume nodulation.

In the developmental sequences of growth substance activity in nodule growth, IAA (A4) emerges as the major *Avena*-active auxin in the nodule. It is present in large quantities at all stages of nodule growth, even until nodules have turned green in early senility. The general effect of this auxin may be one of supra-optimal inhibition of meristem elongation as originally suggested by Thimann (1939). If so, one might expect nodules to elongate if suitably relieved from hyperauxomy early in their growth. This has not been achieved. IAA is the only auxin detectable in very young nodules and it may well be of function in the mitotic stimulation of early nodule development (see Thimann 1955). In this connection high levels of IAA have been recorded from various fungal and bacterial galls and tumours which also exhibit pronounced tissue proliferation unaccompanied by normal cell elongation.

Coincident with nodule pigmentation, two further accelerators become detectable in nodule extracts, one (A1) to remain as a constant feature of tissue extracts, the other (A3) to diminish markedly in subsequent nodule growth. Later in nodule development the two inhibitors, I1 and I2, apparently increase in nodule tissues. What do these fluctuations in minor constituents represent? The well-defined A3 maximum may be singled out as possibly important as a temporary reserve of indole material for subsequent auxin releases in the nodule.

A biochemical approach is obviously required to add further to the present picture. In the first place this might be aimed at discovering by what systems and with what materials the high levels of the various growth substances are elaborated and maintained in the nodule. Secondly, the physiological implications of high levels of indole compounds in the nodule might be examined, particularly with reference to possible interaction with other classes of growth substances, e.g. the kinins and gibberellins. The results from such an approach might well pave the way to further study of the basic problem of how nodule growth is synchronized with general host plant development.

V. Acknowledgments

The work described in this paper forms part of a Ph. D. thesis and I wish to express my thanks to my supervisor, Professor J. Heslop-Harrison, Queen's University, Belfast, for continued interest and suggestions. I am indebted to Mr. B. E. S. Gunning, Queen's University, Belfast, for his assistance with the chromatography techniques. I would also like to thank Dr. R. N. Robertson, Plant Physiology Unit, Division of Food Preservation and Transport, C.S.I.R.O., for valuable help in the preparation of this paper.

VI. References