THE SYMBIOTIC SYNTHESIS OF AUXIN BY LEGUMES AND NODULE BACTERIA AND ITS ROLE IN NODULE DEVELOPMENT

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Summary

When subterranean clover (*Trifolium subterraneum* L.) plants were grown for 3 weeks over distilled water, tryptophan could be detected in the root medium of both sterile cultures and those inoculated with *Rhizobium trifolii* 3 days earlier. Auxin could be detected only in the inoculated medium. The auxin had the chromatographic and growth properties of indole-3-acetic acid (IAA). Since nodule bacteria produce auxin only in the presence of tryptophan, which is a probable precursor of IAA, it is suggested that the tryptophan exuded by clover roots is converted to IAA by nodule bacteria. Auxin was still produced in the root medium when strains of *Rhizobium* which do not nodulate subterranean clover roots were used as inoculant, or when nitrate, which delays nodulation, was present in the medium.

Tryptophan, at high concentrations, delayed nodule initiation in lucerne (*Medicago sativa* L.) plants grown on a mineral salts agar, while α-naphthaleneacetic acid, an auxin, also delayed initiation, and in addition decreased the total number of nodules formed and prevented many plants from forming nodules. An antiauxin, *p*-chlorophenoxy*iso*butyric acid, did not influence nodule initiation, but increased the rate of nodulation and the total number of nodules formed per plant. A root growth promotor, α-(1-naphthylmethylsulphide)-propionic acid did not influence nodule initiation or number. Kinetin inhibited root growth, prevented some plants from nodulating, and reduced the number of nodules formed. Gibberellic acid slightly delayed nodule initiation, but greatly reduced nodule number, while root weight and nodule volume per plant were unchanged. Coconut milk inhibited nodule initiation.

A possible mechanism of root-hair infection and nodule inception is discussed.

I. INTRODUCTION

The detection of an auxin in the nodules of a number of legumes led Thimann (1936, 1939) to postulate a role for auxin in nodule formation and growth. These and all subsequent detections of auxin in legume–nodule bacteria associations were made after the infection process and nodule initiation had occurred (see Pate 1958). In the present investigation the formation of auxin in legume root media (meaning here the media in which legume roots grew), at the time of infection and before nodule initiation, is studied. Also the effects of the presence in legume root media of auxin, auxin precursor, and other growth regulators upon nodule formation are studied.

II. MATERIALS AND METHODS

(a) *Growth of Test Legumes*

All plants were grown under bacteriologically controlled conditions.

(i) *For the Production of Tryptophan and Auxin.*—Seeds of subterranean clover (*Trifolium subterraneum* L., Mount Barker strain) were surface-sterilized and sown

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in 6 by 1 in. glass tubes (five seeds per tube) on moistened pads of glass wool over 10 ml of glass-distilled water or, when required, potassium nitrate solution. Since plants were grown for 21 days only and grew slowly, seed reserves provided ample nutrient supply. After sowing, tubes were held at 4°C for 48 hr to break seed dormancy, then at 26°C in darkness for 48 hr to give maximal germination. After germination they were placed in a louvre-shaded glass-house (Hely 1959). Eighteen days after sowing, the liquid medium was inoculated where necessary. Three days later the seedlings were removed from the tubes and the root medium was collected.

(ii) *For Nodulation Studies.*—Seeds of lucerne (*Medicago sativa* L., Hunter River strain) or barrel medic (*Medicago tribuloides* Desr., strain 173) were surface-sterilized and sown on mineral agar slopes in 6 by $\frac{3}{4}$ in. test tubes using a technique which was essentially that of Thornton (1930). These species were used in nodulation studies because they nodulate more slowly than subterranean clover.

Seeds were germinated under the same conditions as the subterranean clover above, before being placed in the controlled conditions of 12 hr of light at 25°C and 12 hr of darkness at 20°C. Light was provided by fluorescent tubes supplemented with incandescent bulbs; the average intensity outside the seedling-growth tubes was 900 f.c. Four days after sowing, inoculum and, where necessary, 1 ml of solutions of tryptophan, potassium nitrate, a plant growth regulator, or distilled water were added. The growth regulators used were α-naphthaleneacetic acid (NAA), 6-(furfurylamino)-purine (kinetin), gibberellic acid, *p*-chlorophenoxy*iso*butyric acid (PCPIB), coconut milk, and α-(1-naphthylmethylsulphide)-propionic acid (NMSP).

The tryptophan and coconut milk solutions were sterilized by filtration, the others by autoclaving.

Observations on nodulation were made daily and the number of days from sowing to the formation of the first macroscopically visible nodule (nodule initiation) was recorded. At 42 days from sowing the plants were harvested and the total number of nodules on each plant was recorded, and in some cases nodule volume and root dry weight were also measured.

(*b*) *Inoculation of Test Legumes with* Rhizobium

Plants were inoculated from 7-day-old cultures at the rate of approximately 10^6 cells per tube. Strains used were *Rh. trifolii* strain A, *Rh. trifolii* Bart A (a non-infective mutant of strain A), *Rh. meliloti* AH_2 (all from Rothamsted Experiment Station, England), *Rh. meliloti* SU277, *Rh. trifolii* SU297, and *Rh. japonicum* CC705.

Immediately prior to inoculation, streak plates on yeast mannitol agar were made from the root medium and tubes thus shown to be contaminated were excluded from the assays.

(*c*) *Shake Cultures of Nodule Bacteria*

Flasks containing 250 ml of the medium of Norris (1958) with or without 4×10^{-6}M L-tryptophan were inoculated, where necessary, with *Rh. trifolii* SU297 and were shaken at 25°C in darkness for 7 days.

(*d*) *Detection of Tryptophan*

Subterranean clover root medium from inoculated (SU297) and non-inoculated cultures was collected, extracted three times with ether, and the aqueous layer was evaporated to dryness under reduced pressure. The residue was extracted with hot ethanol and the alcohol solution was filtered and evaporated to dryness under reduced pressure. This residue was dissolved in 5 ml of a solution of mineral salts and glucose in distilled water and was sterilized by filtration through sintered glass. Distilled water controls were similarly treated and standards containing 10^{-6}, 3×10^{-6}, 10^{-5}M L-tryptophan were prepared. In early experiments, solutions were inoculated with the tryptophan-requiring mutant *Salmonella typhimurium* strain T_1, and in later experiments with the tryptophan- or indole-requiring mutant *Pseudomonas aeruginosa* strain 1-4 (kindly provided by Dr. B. W. Holloway, University of Melbourne). Any indole present in the root medium was removed by the preliminary ether extraction. Cultures were held at 37°C for 24 hr, then bacterial growth was measured as optical density at 700 mμ using a Unicam spectrophotometer.

(*e*) *Assay for Auxin*

Bulked growth liquid from 30 or more water-culture tubes or shake-culture liquid was brought to pH 3·5 with phosphoric acid, then extracted three times with peroxide-free ether. The ether was evaporated off and the residue dissolved in 2 ml 1·5 per cent. agar. From this, blocks 10 mm^3 were obtained and used in the *Avena* curvature method of auxin assay, carried out exactly as described by Zwar and Rijven (1956).

(*f*) *Chromatography of Auxins*

The residues from the evaporation of ether extracts obtained in the previous section were chromatographed on No. 1 Whatman filter paper. Guide chromatograms of synthetic IAA were also run and the R_F ranges of these guide spots in the solvents used were as follows: *iso*propanol–water–30 per cent. ammonia (10 : 1 : 1 v/v), R_F 0·3–0·45; methanol–water–30 per cent. ammonia (10 : 1 : 1 v/v), R_F 0·5–0·65; and water, R_F 0·85–0·9.

Chromatograms were treated in one of the following ways:

(1) Sprayed with Salkowski or Ehrlich reagents (Stowe and Thimann 1954);

(2) Cut into portions and the auxins in each portion assayed by the *Avena* coleoptile section test (Kefford 1955);

(3) The areas of the chromatograms corresponding with IAA markers were eluted with ethanol, the alcohol evaporated, and the residue dissolved in agar solution for assay in the *Avena* curvature test.

III. Results

(*a*) *Exudation of Tryptophan by Subterranean Clover Roots*

The results in Table 1 show that tryptophan was detected in the concentrated growth medium of both inoculated and uninoculated subterranean clover. These results obtained with *Pseudomonas aeruginosa* strain 1-4 were supported by similar

results obtained with *Salmonella typhimurium* strain T_1. The lower tryptophan content in the inoculated medium was presumably partly due to conversion to IAA by the nodule bacteria.

TABLE 1

OCCURRENCE OF TRYPTOPHAN IN ROOT EXUDATES OF INOCULATED (STRAIN SU297) OR UNINOCULATED SUBTERRANEAN CLOVER, AS MEASURED BY THE GROWTH OF A TRYPTOPHAN-REQUIRING STRAIN OF PSEUDOMONAS AERUGINOSA

Growth is expressed as optical density (at 700 mμ) of the culture and the results of duplicate experiments (I and II) are given

Addition to *Pseudomonas* Medium	Growth of *Pseudomonas* (optical density × 10^3)	Addition to *Pseudomonas* Medium	Growth of *Pseudomonas* (optical density × 10^3)
Tryptophan 10^{-6}M	6	Root exudate	
Tryptophan 3×10^{-6}M	32	SU297 present (I)	5
Tryptophan 10^{-5}M	48	SU297 present (II)	25
Clover growth medium (I)	0	SU297 absent (I)	43
Clover growth medium (II)	0	SU297 absent (II)	46

(*b*) *Production of Auxin by Subterranean Clover and Associated Nodule Bacteria*

Data from tests 1 and 2 in Table 2 show that, when subterranean clover plants were grown alone on distilled water, no auxin was detected in extracts of the root

TABLE 2

AVENA COLEOPTILE CURVATURES GIVEN BY EXTRACTS OF ROOT GROWTH MEDIUM FOLLOWING CULTURE OF SUBTERRANEAN CLOVER PLANTS INOCULATED WITH THE RHIZOBIUM STRAINS INDICATED

The curvatures given by a pure agar control and by IAA at a concentration of $7{\cdot}5 \times 10^{-8}$ g/ml are also shown

Test No.	Inoculant	No. of Tubes Pooled	Curvature by Extract (degrees)	Curvature by Control (degrees)	Curvature by IAA (degrees)
1	Uninoculated	44	− 0·7	+0·3	−22·1
1	Clover strain SU297	44	−28·1	+0·3	−22·1
2	Uninoculated	48	0·0	+1·0	−27·7
3	Clover strain SU297	25	−14·2	0·0	−26·6
4	Clover strain A	82	−25·6	+0·4	−26·1
4	Clover strain Bart A	79	−21·3	+0·4	−26·1
5	Clover strain SU297	44	−20·9	−1·3	−29·0
5	Lucerne strain AH_2	41	−13·9	−1·3	−29·0
5	Soybean strain CC705	28	− 2·0	−1·3	−29·0
6	Soybean strain CC705	46	0·0	−0·7	−29·7

growth medium. If, however, for the last 3 days strain SU297 was present in the culture, auxin could be detected (tests 1, 3, and 5, Table 2). This auxin produced

curvatures at a similar distance down the *Avena* coleoptiles to that given by IAA, showing that it was readily transported. The auxin activity had the same R_F values as IAA in three solvent systems (Table 3) and auxin activity could not be detected on the chromatograms other than in the IAA position.

TABLE 3

AVENA COLEOPTILE CURVATURES GIVEN BY ELUATES OF THE AREAS CORRESPONDING WITH IAA GUIDES AND THE REMAINDER OF CHROMATOGRAMS OF EXTRACTS OF THE ROOT GROWTH LIQUID OF SUBTERRANEAN CLOVER PLANTS INOCULATED WITH STRAIN SU297

The solvents used to develop the chromatograms and curvatures given by a pure agar control and IAA at a concentration of $7{\cdot}5 \times 10^{-8}$ g/ml are also shown

Chromatographic Solvent	Curvature by Eluate of IAA Guides (degrees)	Curvature by Eluate of the Remainder (degrees)	Curvature by Control (degrees)	Curvature by IAA (degrees)
*iso*Propanol–ammonia	−23·2	−0·3	+1·0	−27·7
Methanol–ammonia	−22·2	—	0·0	−26·6
Water	−13·1	−1·8	0·0	−26·6

Extracts of shake cultures of SU297 grown in the presence of tryptophan produced an auxin with the same R_F value as IAA in the solvents *iso*propanol–ammonia and water. When sprayed with the Salkowski or Ehrlich reagents, similar chroma-

TABLE 4

AVENA COLEOPTILE CURVATURES GIVEN BY EXTRACTS OF ROOT GROWTH LIQUIDS WHICH CONTAINED THE CONCENTRATIONS OF POTASSIUM NITRATE SHOWN, AND IN WHICH SUBTERRANEAN CLOVER PLANTS, INOCULATED WITH STRAIN SU297, HAD GROWN

Conventions as in Table 2. The effects of the same concentrations of potassium nitrate upon time to nodulation are also shown

Test No.	Potassium Nitrate Concn. (%)	Days to First Nodule	Number of Tubes	Curvature by Extract (degrees)	Curvature by Control (degrees)	Curvature by IAA (degrees)
5	0·0	4·3	45	−20·9	−1·3	−29·0
5	0·05	8·4	44	−22·4	−1·3	−29·0
6	0·05	8·4	39	−24·8	−0·7	−29·7
6	0·1	8·6	41	−23·5	−0·7	−29·7
6	0·2	9·9	32	−26·6	−0·7	−29·7

tograms held spots of the same colour and R_F value as IAA, and colour was detected only at the IAA position. Using the *Avena* section assay, no IAA could be detected in extracts of shake cultures of SU297 not containing tryptophan.

The non-infective strain, Bart A, produced auxin in the presence of subterranean clover roots to the same extent as the infective parent, strain A (Table 2, test 4). The lucerne strain AH_2 produced auxin in the presence of clover roots (Table 2, test 5), but the soybean strain CC705 did not (Table 2, test 5), even when

TABLE 5

INFLUENCES OF SOME PLANT GROWTH REGULATORS UPON THE NODULATION BEHAVIOUR OF LUCERNE PLANTS INOCULATED WITH STRAIN AH_2

For the treatments in which some plants failed to nodulate, the mean times to nodule initiation and the mean numbers of nodules per plant were calculated on nodulated plants only

Treatment	Concn. (M)	Plants Nodulated (%)	Nodule Initiation (days)	Nodule Number per Plant Nodulated	Nodule Volume (mm^3)		Root Weight (mg)
					Mean per Plant	Mean per Nodule	
Experiment 1							
Control		100	10·8	8·5	9·7	1·14	7·0
NAA	10^{-6}	30	32·0***	3·7***			
NAA	10^{-7}	53	14·8**	5·6**	10·6	1·89	6·2
PCPIB	10^{-5}	100	10·7	12·6***	12·3*	0·98	7·1
PCPIB	10^{-6}	100	11·7	9·8			
Gibberellic acid	10^{-6}	100	13·3**	5·1***	9·6	1·88	6·3
Gibberellic acid	10^{-8}	100	11·4	9·2			
Kinetin	10^{-8}	58	12·8*	10·0			
Kinetin	10^{-9}	100	13·0**	10·0			
Coconut milk	5%	100	13·8***	8·8			
Coconut milk	1%	100	10·8	10·9			
Experiment 2							
Control		100	13·7	12·7			
NAA	10^{-8}	100	12·9	12·7			
Kinetin	10^{-6}	40	32·0***	5·0***			
NMSP	10^{-5}	100	13·2	11·8			
NMSP	10^{-6}	100	14·0	12·7			
NMSP	10^{-7}	100	13·4	13·8			

* Difference from control significant at $P = 0·05$.
** Difference from control significant at $P = 0·01$.
*** Difference from control significant at $P = 0·001$.

plants and bacteria were cultured together for 6 days instead of the usual 3 days (Table 2, test 6). Table 4 shows that the presence of potassium nitrate in the root growth medium delayed nodulation, but did not affect the production of auxin by subterranean clover seedlings and strain SU297.

(c) *Influences of Growth Regulators and Tryptophan on the Nodulation of Lucerne*

In Table 5 are shown the results of two experiments in which growth regulators were added to cultures of lucerne plants inoculated with strain AH_2.

TABLE 6

THE INFLUENCE OF L-TRYPTOPHAN UPON TIME (DAYS) TO THE APPEARANCE OF THE FIRST NODULE ON LUCERNE PLANTS INOCULATED WITH STRAIN AH_2 OR BARREL MEDIC PLANTS INOCULATED WITH STRAIN SU277

Plant	Test No.	Control	L-Tryptophan Concentration (M)									
			$1{\cdot}25 \times 10^{-5}$	10^{-5}	$2{\cdot}5 \times 10^{-6}$	10^{-6}	5×10^{-7}	10^{-7}	2×10^{-8}	10^{-8}	4×10^{-9}	10^{-9}
Lucerne	B	14·9		18·1		15·0		12·4		15·3		14·8
	C	10·1	17·5		15·1		11·9	9·6	10·9			
	E	12·2	19·0		14·2		14·0	12·4	10·7			
	H_1	11·7			16·9		12·7	13·5	12·3		17·8	
Barrel medic	H_2	14·6			14·5		14·3	14·5	12·3		15·6	
	J	9·9						10·1	10·7		10·4	
	K_2	13·7						14·1	12·5		15·3	

The auxin NAA, in concentrations down to 10^{-7}M, caused a delay in the initiation of nodules and a decrease in the number of nodules formed and prevented many plants from forming any nodules. At high concentrations, NAA caused an obvious inhibition of root growth, but at 10^{-7}M the dry weight of roots was the same as controls and the nodule number was still reduced. The volumes of individual nodules on plants treated with 10^{-7}M NAA were larger than the controls and this resulted in the nodule volume per plant being the same for NAA-treated plants and control plants. NAA was used in these tests because IAA is decomposed by light and plant enzymes.

Treatment with the antiauxin PCPIB did not influence nodule initiation, but, at 10^{-5}M, nodule number and nodule volume per plant were increased. The substance NMSP which has been reported to promote root growth (Street 1954), did not influence nodule initiation or number.

Treatment with 10^{-8}M kinetin prevented half of the plants from nodulating, but those that nodulated produced the first nodule at the same time as the controls. Nodule number per nodulated plant was unaffected by kinetin at 10^{-8} or 10^{-9}M. Concentrations of 10^{-6}M or higher caused visible inhibition of the root main axis growth and lateral development. Few such plants nodulated and on those that nodulated, the nodules were fewer and appeared later than on control plants.

Gibberellic acid at 10^{-6}M slightly delayed nodule initiation and greatly reduced nodule number. Root weight and nodule volume per plant, however, were unchanged by this treatment.

Coconut milk at 5 per cent. concentration caused a delay of nodule initiation but did not affect nodule number; 1 per cent. coconut milk produced no effects.

In Table 6 are presented a selection of results of experiments, in which the effects upon nodule initiation of the addition of L-tryptophan to cultures of lucerne inoculated with strain AH_2 and barrel medic inoculated with strain SU277 were observed. At concentrations above 10^{-5}M, tryptophan treatment clearly delayed nodulation. At concentrations of 10^{-7}M or 2×10^{-8}M a slight hastening of nodulation was observed in tests B, C, E, H_2, and K_2 but overall a statistically significant hastening was not established.

IV. Discussion

(*a*) *Symbiotic Synthesis of Auxin*

It has been shown that an auxin is produced when subterranean clover seedlings and nodule bacteria are cultured together, but in the absence of bacteria no auxin is produced. Since nodule bacteria do not produce auxin in the absence of tryptophan (see also Georgi and Beguin 1939) and clover and other legume roots exude tryptophan (Rovira 1956; Dehay and Care 1958), it can be concluded that in the absence of clover roots no auxin would be produced. Hence the presence of both nodule bacteria and clover roots was essential for the production of auxin in the root medium.

(*b*) *Identity of the Auxin*

Although IAA has not been isolated from cultures of nodule bacteria, there is considerable evidence that it is the auxin produced. The biological assay used here, the *Avena* curvature test, involves the polar transport of a substance down the coleoptile for a "deep" curvature to be obtained. The auxin transport system has been shown to be highly specific, in general only IAA or substances readily converted to it being transported (Zwar and Rijven 1956). There is additional chromatographic evidence from extracts of root medium and shake cultures of nodule bacteria. In each of these, the auxin activity had the same R_F value as IAA and activity was detected only at this R_F. On chromatograms of extracts of shake cultures, a substance giving the correct colour reactions was detected at the IAA position.

Assuming the auxin is IAA, its concentration in the root medium of 21-day-old subterranean clover seedlings inoculated for 3 days with SU297 can be roughly estimated as 5×10^{-7} g/l. This value, however, has doubtful significance owing to the destruction of IAA in the root medium by light and perhaps by other means. Tryptophan would be similarly destroyed, thus the rough estimate of its concentration as $7{\cdot}5\times10^{-6}$ g/l in root medium of uninoculated clover seedlings, also has little quantitative significance. The destruction of indole derivatives in the root medium would be accelerated by the presence of substances such as riboflavin and of heavy metals. For the latter reason it is an advantage to be able to grow seedlings in water.

Since it is probable that, as in higher plants (Gordon 1956) and in other micro-organisms (Brian 1957), *Rhizobium* produces IAA from tryptophan, it can be concluded that when clover roots and nodule bacteria are cultured together, tryptophan, exuded by the roots, is converted to IAA by the nodule bacteria.

Tests for growth activity and colour reactions on chromatograms of extracts of *Rhizobium* SU297 shake cultures revealed only one growth-active area and one area giving colour reactions of indole derivatives. Pate (1958) found chromatographically that clover nodules contained three auxins. One of these was probably IAA; the others, according to their R_F values, may have been conjugation compounds of IAA with amino acids or ammonia. Such conjugation compounds are formed in nodules treated with IAA *in vitro* (Andreae, personal communication) and the reactions are considered to be for the detoxification of IAA. If these reactions occur normally in nodules it would be the first demonstration of a natural function for them. Their existence in nodules could also explain the ability of these root tissues to tolerate the abnormally high concentrations of auxin found in nodules, in some respects nodules being physiologically equivalent to lateral roots (Nutman 1956). Of course, other reactions such as oxidation of IAA or the auxin transport system may have roles in localizing the effects of nodule auxin.

(*c*) *Possible Roles for Auxin in Infection Thread and Nodule Growth*

Nutman (1956) and Brian (1957) consider that the role of auxin in nodulation is the curling of root hairs. We suggest that auxin may also play an important

role in the growth of the infection thread and the initiation of the cell division leading to nodule formation. A consideration of these growth processes in the light of the known growth effects of IAA provides support for this suggestion. The sequence of events could be envisaged as follows:

Morphological studies show that the infection thread is a sealed bag inside the root hair and that its wall is of host plant origin (Nutman 1956). Since this bag is extending, pressure inside it must be greater than the pressure inside the root hair. It is postulated that a colony of nodule bacteria on a root hair could do two things: first, it could produce IAA which renders the cell wall plastic; secondly, it could produce osmotic conditions which would cause the root-hair cytoplasm to retreat from the colony. Repetition of these processes, as the bacteria follows the growth of the thread, could account for growth of an infection thread through a root-hair cell. Growth into an adjacent cell could result from the retreat of a neighbouring cytoplasm at a pit area where the cytoplasms are continuous and the cell wall is still capable of being loosened by IAA.

The cell divisions in the root cortex, which give rise to the nodule, could also be initiated by IAA produced by nodule bacteria. For cell division to be initiated in many tissues, a kinin and an auxin are required (Skoog and Miller 1957). Thus cell division could be initiated when the infection thread or actually the IAA produced in it approached a cell containing the required concentration of a kinin. The initial division in nodule formation is by a cell differing from its neighbours in being disomatic.

The above conjecture relates only to the *local* production of IAA at the point of entry of a bacterial colony into a root hair and subsequently in the infection thread. Our results indicate that the production of IAA *generally* in a liquid growth medium of restricted volume does not promote the effects postulated. In this situation IAA appears to inhibit nodule development by virtue of its general inhibitory action on root development. It was hoped that with the addition of graded amounts of tryptophan, a condition might be reached in which localized IAA production at the root-hair surface might be stimulated, before the overall concentration of IAA in the medium reached an inhibitory level. However, only slight and statistically insignificant stimulation was found at any level in the tryptophan concentration series. It is probable that under the culture conditions used, a localized build up in IAA concentration was not possible, or that tryptophan concentration was not limiting infection.

(*d*) *Production of IAA under Conditions Unfavourable to Nodulation*

In two situations where clover plants and nodule bacteria were cultured together, but where nodules would not be formed, IAA was still detected (Table 2). These situations were: (1) clover roots with Bart A, the non-infective mutant of a clover strain, (2) clover roots with lucerne strain AH_2. In a third situation (clover roots with strain SU297 in the presence of nitrate, where nodulation was substantially delayed) IAA was again readily detectable (Table 4). In these cases IAA production cannot be the block in the complex of processes that contribute to nodule formation.

When clover seedlings were cultured with soybean strain CC705, no auxin was detected in the root medium. Also these bacteria were found not to convert tryptophan to IAA in shake culture. Both negative results may result from the slow relative growth of this class of bacteria (Burris and Wilson 1939). The bacteria would appear capable of synthesizing IAA because we have found the nodules of soybean, cowpea, and peanut to contain IAA at approximately 50 mg/kg fresh nodule tissue.

(e) *Effects of Growth Regulators upon Nodulation*

The survey of the effects of the addition of a variety of growth regulators to legume growth media (Table 5) produced no clear leads to roles for these substances in nodulation. But some of the results obtained may be relevant to experiments done under the same conditions of restricted volume of medium, namely preplanting experiments. Nutman (1953, 1957) observed the nodular behaviour of plants grown on media in which other legumes had previously grown. Short periods of preplanting hastened the formation of the first nodule, while longer periods caused a diminution in the rate of formation of subsequent nodules until even fewer nodules were formed than on non-preplanted controls. Our results have shown that preplanting would add tryptophan to the medium and nodule bacteria would convert this to IAA. Also the auxin NAA added to the medium reduces the number of nodules formed, while addition of the antiauxin PCPIB increases this number. We suggest that the number of nodules produced in media of restricted volume is limited by the auxin accumulated under these conditions and that the inhibitory preplanting effects are due to auxin. The observed hastening of nodulation by preplanting has been shown to be at least in part due to the removal of traces of nitrate from the medium (Gibson 1959).

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