

Studies on the Nodule Bacteria. X.  
Influence of Some Stimulating Chemicals  
with Special Reference to the Alkaloids upon  
the Growth and Morphology of  
the Nodule Bacteria.

By

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In this investigation, the influence of ten different alkaloids, three non-alkaloids and yeast water was tested with special reference to the growth and morphology of the nodule bacteria.

Regarding the formation of bacteroids by the nodule bacteria, BELJERINCK<sup>1)</sup> reported in 1888, and many other papers are found since then. BEWLEY and HUTCHINSON<sup>2)</sup> described their life-cycle in the culture medium dividing the cycle into five stage and observed as to the factors which cause the production of each stage. THORNTON and GANGULEE<sup>3)</sup> studied the morphological change of the nodule bacteria in the soil and also in the host plant. LATER GIBSON<sup>4)</sup> noted six different morphological variation of the nodule bacteria<sup>5-9)</sup>. The authors observed the bacteroids frequently in the course of previous investigation. It is very interesting to know at what stage in the life-cycle the nitrogen is fixed in a large quantity, and on this subject BELJERINCK<sup>1)</sup> reported that the fixation takes place only when the bacteroids are present which was substantiated by NOBBE and HILTNER<sup>10)</sup> who experimented with peas and robinie. LATER, however, BELJERINCK's hypothesis was refuted by PFEIFFER and LÖHNIS<sup>11)</sup> although it is not conclusive. On the other hand, the presence of bacteroids in the nodules which are rather rare in the artificial culture, seems to have some connection with the fixation of nitrogen. At the sametime, it was reported that the bacteroids can be produced in the culture medium by changing the composition of medium especially by the addition of alkaloids as noted by BARTHEL<sup>12)</sup> who chaimed that the formation of bacteroids in the nodules is due to their alkaloid contents. Again VITA<sup>13)</sup> and HARTANTIS<sup>14)</sup> reported that the leguminous seeds under aseptic condition fix the nitrogen in the presence of alkaloids but it was refuted by GIRTSCHANOFF<sup>15)</sup>. These reports stimulated the authors's interests so that an enquiry was made here as to the influence of alkaloids and some other chemicals on the nodule bacteria.

## Experimental.

### 1.) Cultures used :

The same strains of nodule bacteria were used as in the previous experiments<sup>5-9</sup>), namely Genge Strain A, B and C, bean and clover. The morphology of these cultures were examined previous to the investigation and are shown in Plate V and VI.

### 2.) Chemicals used :

The chemicals used in this investigation were chiefly those of alkaloids and a few of others which have been known to stimulate the formation of nodules and also the emodine is included since it is found in Habuso (*Cassia occidentalis* L.) and Ebisuso (*C. Tora* L.) which are the leguminous plants without the nodules. The chemicals are listed below :

#### a.) Alkaloids :

Guanidine [ $\text{KHC}(\text{NH}_2)_2$ ], Pyridine [ $\text{CH}(\text{CH}, \text{CH})_2\text{N}$ ]  
 Caffeine [ $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2 \cdot \text{H}_2\text{O}$ ], Strychnine [ $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2$ ]  
 Strychnine nitrate [ $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2\text{HNO}_3$ ], Brucine [ $\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4 \cdot 4\text{H}_2\text{O}$ ]  
 Chinoline [ $(\text{CHCH})_2\text{C}_2\text{CHCHNCH}$ ]  
 Quinine sulfate [ $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2\text{H}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ ]  
 Quinine hydrochloride [ $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2\text{HCl} \cdot 2\text{H}_2\text{O}$ ]  
 Morphine hydrochloride [ $\text{C}_{17}\text{H}_{19}\text{NO}_3\text{HCl} \cdot 3\text{H}_2\text{O}$ ]

#### b.) Non-alkaloids :

Sodium succinate [ $\text{C}_4\text{O}_4\text{H}_4\text{Na}_2$ ], Anthraquinone [ $\text{C}_6\text{H}_4(\text{CO})_2\text{C}_6\text{H}_4$ ]  
 Emodine [ $\text{C}_{14}\text{H}_4\text{O}_2(\text{OH})_3\text{CH}_3$ ]

There are two varieties of emodine, namely aloce modine and frangulaemodine and the emodine found in the Cassia plants belong to the latter and is considered to be the derivative of anthraquinone or trioxymethylanthraquinone.

### 3.) Preparation of culture media :

The medium was prepared after ALLISON<sup>5)</sup> slightly modified and the chemicals were added in different concentrations by taking 1 % solution of each chemical. Some chemicals were not dissolved in water and used as emulsion. The yeast water was made up as usual and added as the original solution. The solid agar medium was made by adding 1.5 % agar as usual.

### 4.) Inoculation and examination of the cultures :

The stock culture of 4 days old, incubated at 28°C. was suspended in the sterile water and the streak culture was made as uniformly as possible, and examined at different intervals for the rate of growth by the naked eyes, and on the 7th day, the microscopical examination was made for the morphology as done previously<sup>5-9</sup>). The results are noted in the following tables.

Table 1.  
Influence of Chemicals on Genge Nodule Bacteria, Strain A.

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Control.	—	+	++	++	++	++	9	Short rod.	$0.3 \times 0.5 - 0.5 \times 0.8$
Guanidine.	1.00	+	+	++	++	++	8	"	$0.3 \times 0.5 - 0.5 \times 1.0$
	0.50	+	+	++	++	++	8	"	"
	0.10	+	++	++	++	++	9	"	$0.3 \times 0.5 - 0.5 \times 0.8$
	0.05	+	+	+	+	+	5	"	$0.3 \times 0.5 - 0.5 \times 1.0$
Pyridine.	1.00	—	—	+	+	+	3	Rod air bubble.	$0.4 \times 0.8 - 1.0 \times 3.5$
	0.50	—	+	++	++	++	7	Short rod.	$0.3 \times 0.5 - 0.6 \times 1.0$
	0.10	—	—	+	++	++	5	"	"
	0.05	—	—	+	++	++	5	Rod granule.	$0.3 \times 0.5 - 0.6 \times 1.5$
Caffeine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	+	+	3	Irregular.	$0.4 \times 0.8 - 1.2 \times 7.0$
	0.05	—	—	+	++	++	5	Rod air bubble.	$0.5 \times 1.0 - 1.0 \times 2.5$
Strychnine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	++	++	5	Rod club granule.	$0.4 \times 0.8 - 1.0 \times 2.0$
	0.05	—	+	+	+	++	5	Short rod.	$0.3 \times 0.6 - 0.7 \times 1.0$
Strychnine nitrate.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	++	++	5	Irregular.	$0.4 \times 0.8 - 1.1 \times 4.0$
	0.05	—	+	++	++	++	10	Rod granule.	$0.3 \times 0.7 - 0.8 \times 2.0$
Brucine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	+	+	++	++	6	Short rod.	$0.3 \times 0.4 - 0.4 \times 0.8$
Chinoline.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	—	—	—	—	0	—	—

Table 1. (Continued.)

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Chinine sulfate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	+	+	+	3	Rod.	$0.3 \times 0.6 - 0.6 \times 1.5$
Chinine hydrochloride.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	-	-	-	0	-	-
Morphine hydrochloride.	1.00	+	+	+	+	++	6	Short rod.	$0.3 \times 0.8 - 0.7 \times 1.0$
	0.50	+	+	+	+	++	6	Rod coccic.	$0.3 \times 0.3 - 0.5 \times 1.2$
	0.10	+	+	+	+	++	6	Rod.	$0.2 \times 0.5 - 0.6 \times 1.2$
	0.05	-	+	+	+	++	5	Short rod.	$0.3 \times 0.6 - 0.5 \times 1.0$
Sodium succinate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	+	+	++	4	Short rod.	$0.3 \times 0.8 - 0.5 \times 1.0$
	0.05	+	+	+	+	++	6	Rod granule.	$0.3 \times 0.8 - 0.5 \times 2.0$
Anthraquinone.	1.00	+	+	+	+	++	6	Short rod.	$0.3 \times 0.5 - 0.5 \times 1.0$
	0.50	+	+	+	+	+	5	"	$0.3 \times 0.5 - 0.4 \times 0.8$
	0.10	+	++	++	++	++	9	Rod coccic.	$0.3 \times 0.4 - 0.5 \times 1.2$
	0.05	-	+	++	++	++	7	Short rod coccic.	$0.3 \times 0.3 - 0.5 \times 1.0$
Emodine.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	+	+	++	++	++	8	Short rod.	$0.2 \times 0.4 - 0.4 \times 0.8$
	0.05	-	+	+	+	++	5	Rod.	$0.4 \times 0.6 - 0.6 \times 1.5$
Yeast water.	10.00	+	++	+++	+++	++++	19	Rod granule.	$0.3 \times 0.5 - 0.8 \times 1.5$
	1.00	+	++	+++	+++	+++	16	"	$0.3 \times 0.5 - 0.8 \times 1.3$
	0.50	+	++	+++	+++	+++	15	"	$0.3 \times 0.5 - 0.8 \times 1.4$
	0.10	+	++	+++	+++	+++	12	"	$0.3 \times 0.5 - 0.8 \times 1.5$
	0.05	+	++	+++	+++	+++	12	"	$0.3 \times 0.6 - 0.7 \times 1.5$

Note: The number of + indicates the rate of growth.

As Table 1 indicates, no growth was found where chinoline and chinine hydrochloride were added; in brucine and chinine sulfate, the feeble growth was obtained only in 0.05% while no growth was observed in 0.5% caffeine, strychnine, strychnine nitrate, sodium succinate, emodine; the best growth was obtained in 10% yeast extract which gave good results in all the cases; the growth in guanidine, anthraquinone was fairly good but no better than that in the control. Morphologically the bacteroids were rather numerous especially in pyridine, 0.1 and 0.5% caffeine and 0.1% strychnine nitrate. They were crossed, y-shaped and some were branched as shown in photographs, Plate VII. ALMON<sup>16</sup> reported that the bacteroids do not rejuvenate but in our cases, they rejuvenated as soon as they were transplanted to yeast mannit agar and resumed the rod forms.

The results obtained with Genge nodule bacteria, strain B are presented in Table 2.

(See Table 2 on page 58-59.)

As noted in Table 2, the complete prohibition of growth took place only in chinoline, and in brucine, chinine sulfate and chinine hydrochloride, grew only in 0.05% concentration while no growth was observed in the higher concentrations than 0.5% in cases of caffeine, strychnine, strychnine nitrate, sodium succinate and emodine; 10% yeast extract was very good as usual, and the others were just about the same as the control. The rod forms were prevalent and a few of the coccic and oval forms were present, and the bacteroids were found in 1% pyridine, 0.10% caffeine and 0.05% chinine sulfate.

Table 3 presents the results obtained with Genge nodule bacteria, strain C.

(See Table 3 on page 60-61.)

No growth was observed in chinoline, chinine sulfate and only in 0.05% chinine hydrochloride, the growth took place while no growth above 0.5% concentration of caffeine, brucine, strychnine nitrate and sodium succinate; in case of emodine, 1.0% concentration alone prevented the growth; the yeast extract was effective as usual. Morphologically a majority of cells were rod and the coccic forms were rather numerous, and some interesting bacteroids were found in caffeine and chinine hydrochloride.

The results obtained with the bean nodule bacteria are noted in Table 4.

(See Table 4 on page 62-63.)

As noted in Table 4, no growth took place in chinoline and chinine sulfate while only 0.05% brucine and chinine hydrochloride allowed the growth; in pyridine, caffeine, strychnine nitrate, sodium succinate and emodine, above 0.5% concentration prohibited the growth; in guanidine and strychnine grew in all the concentrations except 1.0%; the yeast extract was as effective as usual. Morphologically the rod forms were prevalent and numerous bacteroids were found in 0.05% pyridine, 0.1% caffeine and 0.1% strychnine nitrate.

Further the results obtained with the clover nodule bacteria, are presented in Table 5. (See Table 5 on page 64-65.)

Table 2.  
Influence of Chemicals on Genge Nodule Bacteria, Strain B.

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Control.	—	+	+	++	++	++	8	Rod.	0.3×0.5-0.6×1.2
Guanidine.	1.00	+	+	+	++	++	7	Short rod.	0.3×0.4-0.6×1.0
	0.50	-	+	++	++	++	7	Short rod coccic.	0.3×0.5-0.6×1.0
	0.10	+	++	++	++	++	9	Short rod.	0.2×0.5-0.5×1.0
	0.05	+	++	++	++	++	9	Short rod coccic.	0.2×0.4-0.5×1.0
Pyridine.	1.00	-	-	+	+	++	4	Irregular air bubble.	0.5×1.0-1.0×5.0
	0.50	+	++	++	++	++	9	Rod granule.	0.3×0.5-0.7×1.2
	0.10	-	+	++	++	++	7	Short rod.	0.3×0.6-0.6×1.0
	0.05	-	+	++	++	++	7	Rod coccic.	0.2×0.3-0.5×1.2
Caffeine.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	+	+	+	3	Irregular air bubble.	0.5×1.2-1.2×4.0
	0.05	-	+	++	++	++	7	Rod granule.	0.4×0.8-0.8×2.0
Strychnine.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	+	+	+	+	4	Rod granule.	0.3×0.5-0.6×1.5
	0.05	-	+	++	++	++	7	"	"
Strychnine nitrate.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	+	+	+	3	Rod granule.	0.3×0.6-0.8×1.8
	0.05	-	+	++	++	++	7	Rod.	0.3×0.5-0.6×1.2
Brucine.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	-	-	-	0	—	—
	0.05	-	+	+	++	++	6	Short rod.	0.3×0.5-0.6×1.0
Chinoline.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	-	-	-	0	—	—
	0.05	-	-	-	-	-	0	—	—

Table 2. (Continued.)

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Chinine sulfate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	-	+	+	2	Irregular.	0.5×1.0-1.0×4.0
Chinine hydrochloride.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	+	+	+	3	Rod.	0.3×0.6-0.5×1.2
Morphine hydrochloride.	1.00	-	+	+	++	++	6	Short rod.	0.3×0.5-0.5×1.0
	0.50	+	+	+	++	++	7	Rod.	0.2×0.5-0.6×1.2
	0.10	-	+	+	++	++	6	"	0.3×0.6-0.5×1.2
	0.05	+	++	++	++	++	9	Short rod.	0.3×0.5-0.6×1.0
Sodium succinate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	+	+	2	Rod granule.	0.3×0.5-0.7×2.0
	0.05	+	+	+	+	+	5	Short rod.	0.3×0.5-0.5×1.0
Anthraquinone.	1.00	-	+	+	+	+	4	"	"
	0.50	+	+	+	+	+	5	"	0.3×0.6-0.5×1.0
	0.10	-	+	+	+	+	4	Irregular air bubble.	0.5×1.0-0.8×2.5
	0.05	-	+	+	+	+	4	Short rod coccic.	0.2×0.3-0.5×1.0
Emodine.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	+	++	++	++	7	Rod.	0.2×0.5-0.6×1.2
	0.05	-	+	+	+	+	4	Rod granule.	0.3×0.5-0.7×1.8
Yeast water.	10.00	+	###	###	###	###	21	Rod coccic granule.	0.3×0.5-0.5×1.5
	1.00	+	###	###	###	###	20	Rod granule.	0.3×0.5-0.7×1.2
	0.50	+	###	###	###	###	20	Rod coccic granule.	0.2×0.5-0.8×1.5
	0.10	+	###	###	###	###	16	Rod oval.	0.3×0.5-0.5×1.5
	0.05	+	###	###	###	###	13	"	0.3×0.5-0.6×1.2

Note: The number of + indicates the rate of growth.

Table 3.  
Influence of Chemicals on Genge Nodule Bacteria, Strain C.

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Control.	—	—	++	++	++	++	8	Short rod coccic.	0.2×0.3-0.5×1.0
Guanidine.	1.00	+	+	++	++	++	8	"	"
	0.50	—	+	++	++	++	7	Coccic short rod.	0.3×0.3-0.5×0.8
	0.10	—	+	+	+	++	5	Short rod coccic.	0.3×0.3-0.5×1.0
	0.05	—	++	++	++	++	8	"	0.3×0.3-0.5×0.8
Pyridine.	1.00	—	—	+	+	+	3	Rod granule.	0.2×0.3-1.0×1.5
	0.50	—	++	++	++	++	8	Rod.	0.3×0.5-0.6×1.2
	0.10	—	++	++	++	++	8	Rod coccic.	0.3×0.3-0.7×1.0
	0.05	—	++	++	++	++	8	"	0.3×0.5-0.5×1.0
Caffeine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	+	+	3	Irregular air bubble.	0.4×0.7-1.0×3.0
	0.05	—	+	+	+	+	4	"	0.3×0.6-0.7×3.0
Strychnine.	1.00	—	—	+	+	+	3	"	0.3×0.5-1.0×2.5
	0.50	—	—	+	+	+	3	Short rod.	0.3×0.5-0.5×1.0
	0.10	—	+	+	+	+	4	Rod.	0.3×0.4-0.7×2.0
	0.05	—	+	+	+	+	4	Short rod coccic.	0.3×0.3-0.5×1.0
Strychnine nitrate.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	+	++	++	++	7	Rod coccic.	0.3×0.3-0.7×1.2
	0.05	—	++	++	++	++	8	Short rod.	0.3×0.4-0.5×1.0
Brucine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	+	+	3	Rod granule.	0.3×0.6-1.0×1.5
	0.05	—	+	+	+	+	4	Coccic short rod.	0.2×0.2-0.5×0.8
Chinoline.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	—	—	—	—	0	—	—



Table 3. (Continued.)

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Chinine sulfate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	-	-	-	0	-	-
Chinine hydrochloride.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	+	+	+	3	Irregular air bubble.	0.5×0.8-1.2×3.0
Morphine hydrochloride.	1.00	-	+	+	+	+	4	Short rod coccic.	0.2×0.3-0.5×1.0
	0.50	-	++	++	++	++	8	"	0.2×0.3-0.5×0.8
	0.10	-	++	++	++	++	8	"	0.3×0.4-0.7×1.0
	0.05	-	++	++	++	++	8	Rod.	0.3×0.4-0.7×1.2
Sodium succinate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	+	++	++	5	Short rod coccic.	0.3×0.4-0.5×0.8
	0.05	-	+	++	++	++	7	Rod granule.	0.3×0.6-1.0×2.0
Anthraquinone.	1.00	-	+	+	+	+	4	Short rod.	0.2×0.5-0.5×0.8
	0.50	-	+	++	++	++	7	Coccic short rod.	0.2×0.3-0.6×1.0
	0.10	-	++	++	++	++	8	Short rod.	0.3×0.5-0.3×1.0
	0.05	-	+	++	++	++	7	Short rod coccic.	0.3×0.5-0.5×1.0
Eminodine.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	+	+	2	Rod club granule.	0.3×0.5-0.6×2.0
	0.10	-	+	+	+	+	4	Rod granule.	0.2×0.3-0.8×2.0
	0.05	-	+	+	+	+	4	Short rod coccic.	0.2×0.3-0.5×0.8
Yeast water.	10.00	-	###	###	###	###	19	Short rod.	0.3×0.6-0.6×0.8
	1.00	-	###	###	###	###	18	"	0.3×0.5-0.7×1.0
	0.50	+	###	###	###	###	17	"	0.2×0.5-0.5×0.8
	0.10	+	###	###	###	###	13	"	0.3×0.5-0.6×1.0
	0.05	-	++	++	++	++	8	"	0.3×0.4-0.5×0.8

Note: The number of + indicates the rate of growth.

Table 4.  
Influence of Chemicals on Bean Nodule Bacteria.

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Control.	—	—	+	++	++	++	7	Rod.	0.3×0.5-0.6×1.2
Guanidine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	+	+	+	+	4	Short rod.	0.3×0.5-0.5×1.0
	0.10	+	+	++	++	++	8	Rod granule.	0.3×0.8-0.6×1.2
	0.05	—	+	+	+	+	4	Short rod.	0.3×0.5-0.5×1.0
Pyridine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	+	++	++	++	7	Rod granule.	0.2×0.4-0.6×1.2
	0.05	—	+	++	++	++	7	Irregular air bubble.	0.3×0.8-1.2×3.5
Caffeine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	+	+	3	Irregular air bubble.	0.5×1.0-1.2×5.5
	0.05	—	+	++	++	++	7	Rod granule.	0.2×0.5-0.8×1.2
Strychnine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	+	+	2	Rod.	0.3×0.5-0.7×1.5
	0.10	—	—	+	++	++	5	Rod club granule.	0.3×0.5-1.0×2.0
	0.05	—	+	+	+	+	4	Short rod.	0.3×0.4-0.6×1.0
Strychnine nitrate.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	+	++	++	5	Irregular air bubble.	0.5×1.0-1.0×5.0
	0.05	—	—	+	++	++	5	Rod granule.	0.3×0.7-0.7×1.2
Brucine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	+	+	+	+	4	Rod coccic.	0.2×0.3-0.5×1.2
Chinoline.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	—	—	—	—	0	—	—

Table 4. (Continued.)

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Chinine sulfate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	-	-	-	0	-	-
Chinine hydrochloride.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	-	-	-	0	-	-
	0.05	-	-	+	+	+	3	Rod club.	0.3×0.5-0.7×2.0
Morphine hydrochloride.	1.00	-	+	++	++	++	7	Rod granule.	0.3×0.5-0.8×1.2
	0.50	-	+	++	++	++	7	Short rod granule.	0.3×0.4-0.5×0.8
	0.10	-	+	++	++	++	7	Short rod.	0.3×0.5-0.6×1.0
	0.05	-	+	++	++	++	7	Rod.	0.2×0.5-0.8×1.5
Sodium succinate.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	-	+	+	+	3	Short rod.	0.3×0.4-0.5×0.8
	0.05	-	+	+	+	+	4	Rod coccic.	0.3×0.4-0.6×1.2
Anthraquinone.	1.00	-	+	+	+	+	4	Short rod.	0.2×0.5-0.5×1.0
	0.50	-	+	+	+	+	4	"	0.3×0.5-0.5×0.8
	0.10	-	+	+	+	+	4	"	0.2×0.3-0.5×1.0
	0.05	-	+	+	+	+	4	"	0.3×0.5-0.5×0.8
Emodine.	1.00	-	-	-	-	-	0	-	-
	0.50	-	-	-	-	-	0	-	-
	0.10	-	+	+	++	++	6	Rod.	0.2×0.5-0.5×1.2
	0.05	-	+	+	+	+	4	"	0.3×0.5-0.6×1.5
Yeast water.	10.00	+	###	###	###	###	20	Rod granule.	0.3×0.6-0.6×1.5
	1.00	+	###	###	###	###	20	"	0.3×0.5-0.9×1.5
	0.50	+	###	###	###	###	20	"	0.3×0.5-0.7×1.5
	0.10	-	##	##	##	##	12	Rod.	0.4×0.7-0.7×1.5
	0.05	-	++	++	++	++	8	"	0.2×0.7-0.7×1.2

Note: The number of + indicates the rate of growth.

Table 5.  
Influence of Chemicals on Clover Nodule Bacteria.

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Control.	—	+	≡	≡	≡	≡	13	Rod.	0.3×0.5-0.6×1.2
Guanidine.	1.00	-	-	+	≡	≡	7	Irregular air bubble.	0.5×0.8-1.1×3.0
	0.50	-	++	++	++	++	8	"	0.3×0.5-0.8×3.0
	0.10	+	++	≡	≡	≡	12	"	0.5×1.0-0.8×3.0
	0.05	+	≡	≡	≡	≡	13	Rod air bubble.	0.3×0.5-0.8×2.5
Pyridine.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	+	++	++	5	Rod.	0.3×0.5-0.7×1.8
	0.10	-	+	++	++	++	7	Rod granule.	0.3×0.5-0.7×1.2
	0.05	-	++	≡	≡	≡	11	Rod club granule.	0.3×0.6-1.0×2.0
Caffeine.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	+	+	+	3	Rod air bubble.	0.3×0.8-1.0×2.5
	0.05	+	+	++	≡	≡	10	"	0.3×0.7-0.7×3.0
Strychnine.	1.00	-	-	++	≡	≡	8	Rod granule.	0.3×0.5-0.7×1.5
	0.50	-	++	≡	≡	≡	11	"	0.3×0.5-0.6×2.0
	0.10	+	++	≡	≡	≡	12	"	0.3×0.5-0.8×2.0
	0.05	+	++	++	++	≡	10	"	0.2×0.7-0.6×1.5
Strychnine nitrate.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	+	≡	≡	≡	≡	13	Rod granule.	0.3×0.5-0.7×1.5
	0.05	++	≡	≡	≡	≡	14	Rod air bubble.	0.3×0.5-0.5×2.5
Brucine.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	-	-	-	0	—	—
	0.05	+	++	≡	≡	≡	12	Rod granule.	0.3×0.5-0.8×2.0
Chinoline.	1.00	-	-	-	-	-	0	—	—
	0.50	-	-	-	-	-	0	—	—
	0.10	-	-	-	-	-	0	—	—
	0.05	-	-	-	-	-	0	—	—

Table 5. (Continued.)

Chemicals.	Conc. (%)	Rate of growth by days.						Cells on 7th day.	
		2	4	7	10	14	Sum of +.	Form.	Size. ( $\mu$ )
Chinine sulfate.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	—	—	—	—	0	—	—
Chinine hydrochloride.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	—	—	—	—	0	—	—
	0.05	—	—	+	+	+	3	Rod granule.	0.3×0.7—0.8×2.0
Morphine hydrochloride.	1.00	+	++	+++	+++	+++	12	Rod club air bubble.	0.3×0.8—0.8×2.5
	0.50	+	++	++	++	++	9	Rod air bubble.	0.4×0.7—1.0×2.5
	0.10	+	++	++	++	+++	10	"	0.3×0.5—0.8×2.5
	0.05	+	+	++	++	+++	9	"	0.3×0.9—0.8×2.5
Sodium succinate.	1.00	—	—	—	—	—	0	—	—
	0.50	—	—	—	—	—	0	—	—
	0.10	—	+	+++	+++	+++	12	Short rod.	0.4×0.5—0.6×1.0
	0.05	—	+	+++	+++	+++	11	Rod.	0.3×0.8—0.6×1.2
Anthraquinone.	1.00	—	++	++	++	+++	9	"	0.3×0.7—0.7×2.0
	0.50	+	+++	+++	+++	+++	13	Rod granule.	"
	0.10	—	++	+++	+++	+++	11	"	"
	0.05	—	+++	+++	+++	+++	12	"	0.5×0.8—0.8×2.0
Emodine.	1.00	—	—	—	—	—	0	—	—
	0.50	—	+	+	++	++	6	Rod granule.	0.3×0.5—0.8×1.5
	0.10	+	++	++	++	+++	10	"	0.3×0.5—0.7×1.5
	0.05	+	++	++	+++	+++	11	"	0.3×0.7—0.7×2.0
Yeast water.	10.00	++	+++	+++	+++	+++	21	"	0.4×0.8—0.8×1.8
	1.00	++	+++	+++	+++	+++	18	Rod.	0.3×0.5—0.7×1.2
	0.50	++	+++	+++	+++	+++	16	"	0.3×0.5—0.5×1.2
	0.10	+	+++	+++	+++	+++	15	Short rod coccic.	0.3×0.4—0.5×0.8
	0.05	+	+++	+++	+++	+++	15	Rod.	0.3×0.8—0.8×1.5

Note: The number of + indicates the rate of growth.

It is noted in Table 5 that chinoline and chinine sulfate prohibited the growth completely while brucine and chinine hydrochloride allowed the growth only in 0.05 % ; no growth above 5 % concentration of caffeine, strychnine nitrate and sodium succinate took place ; in pyridine and emodine, no growth was possible only in 1.0 %. As a whole, the growth of clover nodule bacteria was about the same as the foregoing organisms, no chemicals added stimulated the growth but the yeast extract. The form of cells was rod mostly, and the bacteroids were numerous in guanidine and caffeine.

The results obtained in the foregoing five experiments seem to indicate that these five different nodule bacteria showed a similar tendency with very slight variation. Besides the yeast extract, guanidine, morphine hydrochloride and anthraquinone have stimulating influence to some extract, while pyridine and strychnine were none better than the control. On the other hand, chinoline depressed entirely the growth of all five strains of nodule bacteria, and chinine as well as brucine were detrimental for the growth. In the previous publication<sup>7)</sup>, it was reported that the alkaloid-like constituents in the nodules have little to do with the growth of nodule bacteria, and now the report was substantiated by the results obtained in this investigation by using the known chemicals. Morphologically no uniform results were obtained, and more or less number of bacteroids were found in all the cases although there were some differences by the different chemicals added, for instance, more of the large bacteroids were formed in caffeine and some in pyridine, strychnine, strychnine nitrate and chinine. In a majority of cases, more bacteroids were produced where the growth was feeble but not all the cases. Caffeine and pyridine seemed to stimulate the formation of bacteroids of large size which are rarely found under the natural conditions, as shown in Plate VI.

The concentrations of chemicals in which comparatively large bacteroids were formed, are given in Table 6. (See Table 6 on next page.)

Table 6 indicates clearly that caffeine stimulates the formation of large bacteroids in all strains of bacteria. It is worthy to note that guanidine causes the formation of large bacteroids with bean nodule bacteria only while morphine hydrochloride has the same action with clover nodule bacteria alone, in all concentrations ; also it is interesting to find that emodine showed some influence.

### Summary.

The influence of the different alkaloids, three non-alkaloids and yeast water on the growth and morphology of the nodule bacteria as investigated and the results are summarized as follows :

- 1.) None of the chemicals tested was effective in stimulating the growth of bacteria in comparison with the yeast water. They in general were rather harmful and more so as the concentration of the chemicals increased.

Table 6.  
Influence of Various Concentration of Chemicals on the Formation  
of large Bactroid.

Chemicals.	Concentration of chemicals (%) and strains of bacteria.				
	Genge A.	Genge B.	Genge C.	Bean.	Clover.
Guanidine.	—	—	—	1.00 0.05 0.10 0.05	—
Pyridine.	1.00	1.00	—	0.05	0.05
Caffeine.	0.10 0.05	0.10 0.05	0.10 0.05	0.10	0.10 0.05
Strychnine.	0.10	—	1.00	0.10	—
Strychnine nitrate.	0.10	—	—	0.10	0.05
Chinine sulfate.	—	0.05	—	—	—
Chinine hydrochloride.	—	—	0.05	0.10	—
Morphine hydrochloride.	—	—	—	—	1.00 0.50 0.10 0.05
Sodium succinate.	—	0.10	0.05	—	—
Emodine.	—	—	0.50 0.10	—	—

Note: The numerals in the table denotes the concentration of chemicals.

2.) Among the chemicals used, guanidine, morphine hydrochloride and anthraquinone were not harmful for the growth ; pyridine and strychnine were not so good as the formers ; chinoline was harmful and the growth of all five strains of bacteria was prohibited, while chinine compounds and brucine were less harmful.

3.) By the addition of chemicals, various forms of bacteria are produced such as rod, coccic, oval, club, branching etc., and in some cases, the granules and vacuoles are produced in the cells. But no correlation was found between the concentration of chemicals and the degree of morphological variation.

4.) Caffeine seemed to be the most effective agent in production of large bacteroids in all strains of bacteria ; pyridine, strychnine, strychnine nitrate and chinine compounds were effective in all cases while guanidine and morphine hydrochloride were effective only against some special bacterial strain. Among the non-alkaloids, the large bacteroids were found where sodium succinate and emodine were added, and none in cases of brucine, anthraquinone and yeast water.

5.) As a whole, more of the large bacteroids were found where the growth was poor but not all the cases.

6.) From the foregoing results, it may be stated that the alkaloids have no stimulating effect on the growth of nodule bacteria but they are effective in producing the large bacteroids.

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### Literature.

- 1.) BEIJERINCK, M. W., *Botanische Zeitung*, 46 : 726, 741, 757, 781, 1888.
- 2.) BEWLEY, W. F., and HUTCHINSON, H. B., *Jour. Agri. Sci.*, 10 : 144, 1920.
- 3.) THORNTON, H. G. and GANGULEE, N., *Proc. Roy. Soc., London, B*, 99 : 427, 1926.
- 4.) GIBSON, T., *Jour. Agri. Sci.*, 18 : 76, 1928.
- 5.) ITANO, A. and A. MATSUURA, *Nōgaku Kenkyū*, 24 : 193, 1935.
- 6.) *Ibid.* 25 : 423, 1935.
- 7.) *Ibid.* 26 : 370, 1936.
- 8.) *Ibid.* *J. Science Soil Manure, Japan*, 12, 1936.
- 9.) *Ibid.* *J. Agr. Chem. Soc., Japan*, 12, 1936.
- 10.) NOBBE, F. und HILTNER, L., *Landw. Vers. Stat.*, 42 : 459, 1893.
- 11.) JÖHNIS, M. P., *Zentbl. Bakt.*, II, 80 : 342, 1930.
- 12.) BARTHEL, C., *Ann. Inst. Pasteur*, 35 : 634, 1921.
- 13.) VITA, N., *Biochem. Ztschr.*, 252 : 278, 1932.
- 14.) HARITANTIS, B. J., *Zitschr. Pfl. Düng. Bodenk., A*, 34 : 257, 1934.
- 15.) GIRTSCHANOFF, K., *Zentbl. Bakt.*, II, 92 : 349, 1935.
- 16.) ALMON, L., *Zentbl. Bakt.*, II, 87 : 289, 1933.

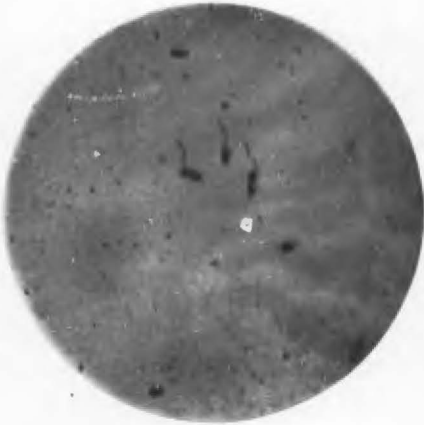


PLATE V.

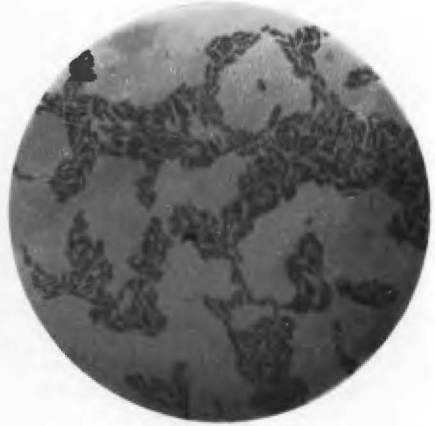
Morphology of Genge Nodule Bacteria at different Age on Yeast-mannit Agar.

(1,000×)

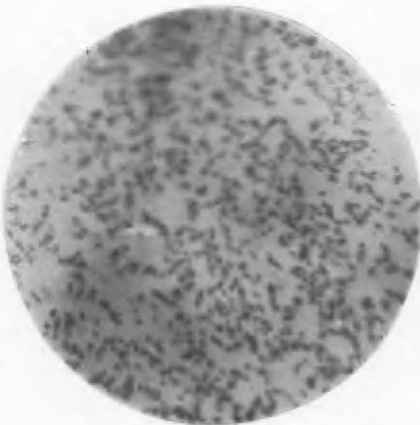
24 hours old.



4 days old.



30 days old.



70 days old.

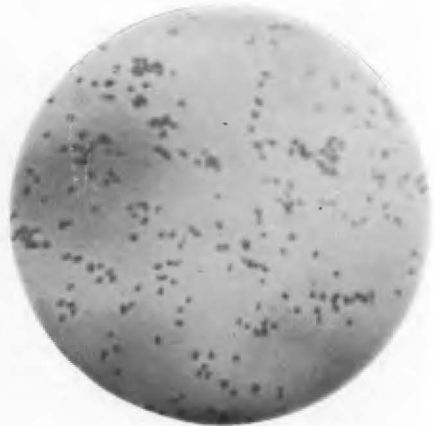
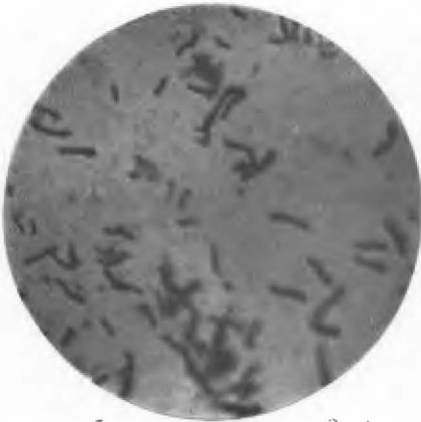


PLATE VI.

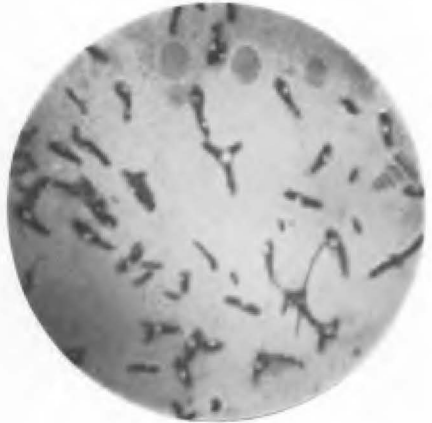
Morphology of Genge Nodule Bacteria in the Nodules on the Plant  
Root of different Age.

(1,000×)

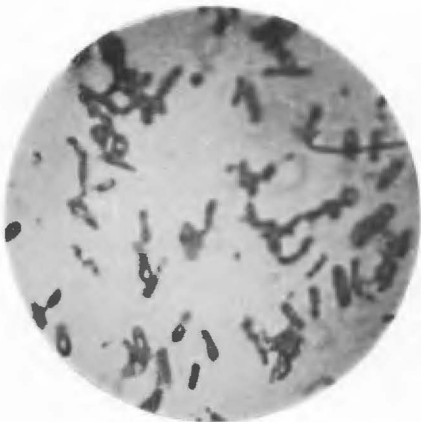
On one leaf stage.



One two leaves stage.



One month old.



Six months old.



PLATE VII.

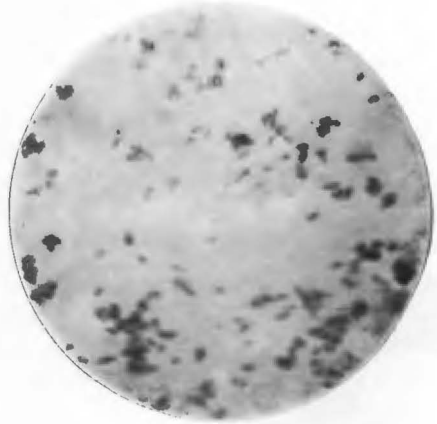
Morphology of Genge Nodule Bacteria with different Chemicals.

(1,000×)

Caffeine 0.10 %.



Caffeine 0.05%.



Strychnine nitrate 0.15 %.



Emodine 0.05 %.

