

Studies on the Nodule Bacteria. IX.
On the Electrical Properties of the Accessory
Substance.

By

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In the previous papers^{1), 2)}, it was reported that the stimulation of the growth of nodule bacteria is due to the organic substances which are soluble in water, alcohol and chloroform, especially eighty to ninety percent of it is extracted by the combined action of water and alcohol. On the other hand, the inorganic substances have no action. In this investigation, an enquiry was made to ascertain the electrical nature of accessory substance so that further knowledge may be obtained regarding the physical properties of substance causing the stimulation.

Experimental.

Electro-dialysis :

Five grams of bean nodules are powdered and placed in the dialysing chamber after KONDO³⁾, using well washed cellophane paper at the anode and sulfuric acid treated paper at the cathode with 110 V, D. C., and dialysed until the electric current decreased gradually and no further change took place.

The investigation was carried out in two stages as follow :

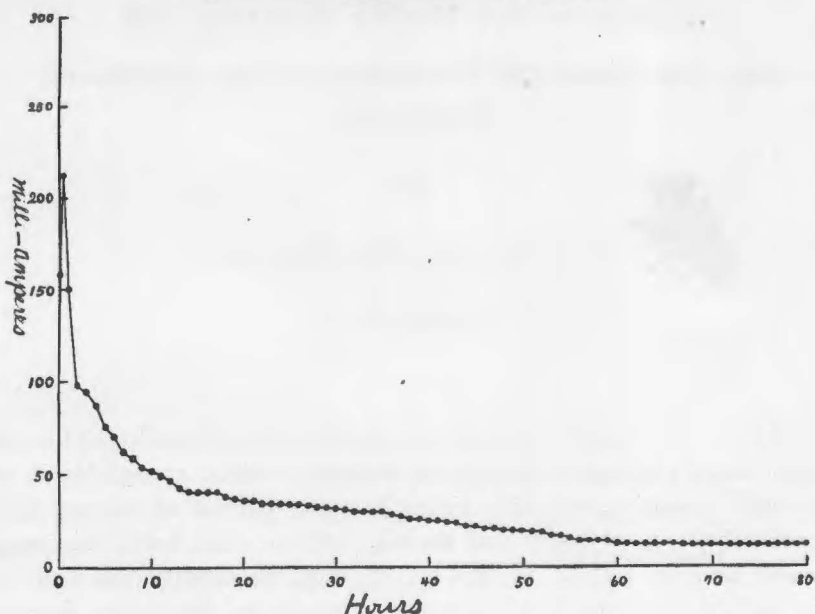
I. *Dialytic nature of accessory substance in the nodules :*

The residue in the dialytic chamber was taken and the culture medium was prepared to test the rate of growth. During the dialysis, the water was changed every hour and the chambers were cooled under running water to prevent the sudden change of temperature. The change of current in the course of dialysis is shown in Fig. 1.

(See Fig. 1 on next page.)

As noted in the figure, the current decreased rapidly during the first thirty minutes which was 212 m.a., and 100 m.a. after two hours, and thence the decline became slow and no further change after 60 hours remaining 12 m.a. at the end of experiment. Then the content in the middle chamber was taken out and

Fig. 1.
Change of Amperage during the Dialysis.



filtered of which the residue was dried and the filtrate was used as the solution. These components were added to the culture medium in proportion of 1 percent of the original sample to test their effect. The results are presented in Table 1.

(See Table 1 on next page.)

Table 1 indicates, 1% original nodule was most effective, and the residue as well as the filtrate from dialysis were beneficial although they were not so good as the yeast extract while much better than the control. It is indicated therefore that by the dialysis not all the accessory substances are removed especially in the residue, and comparatively a large amount is left undialysed. Considering these results in the light of a preceding paper, it is reasonable to suppose that the accessory action is brought about by the organic substance since the inorganic constituents are removed by dialysis to a large extent.

II. *Electrical nature of the accessory substance in the nodules.*

To determine the electrical nature of the accessory substance in the nodules, the extracts of the nodules were prepared by using cold and hot water and alcohol. Then the extracts were dialysed separately by the method just described for 24 hours. In the course of dialysis, the water in the anodic and cathodic chambers was changed after six and twelve hours, and after dialysis, the one sample was taken out from each the anodic, cathodic and middle chamber and also from the residue, and the tests were carried out as described previously.

Table 1.
Influence of Dialysed Nodules on the Growth of Nodule Bacteria.

Nodule bacteria.	Substance added.	Rate of growth by days.				Sum of +.	Wt. of bacterial cells on 7th day.
		2	4	7			
Genge Strain A.	Control. . . .	+	++	++	5	(mg.) 0.5	
	Yeast.	++	+++	+++	9	11.8	
	Nodule (1%). .	++	+++	+++	11	14.8	
	Dialysed nodule.	+	++	++	6	2.9	
	Dialysed filtrate.	+	++	++	7	3.4	
Genge Strain B.	Control. . . .	-	+	+	2	2.5	
	Yeast.	+	++	+++	9	13.1	
	Nodule (1%). .	+	+++	+++	10	13.9	
	Dialysed nodule.	+	++	++	8	9.8	
	Dialysed filtrate.	+	+	++	5	2.8	
Genge Strain C.	Control. . . .	++	++	++	6	0.4	
	Yeast.	++	+++	+++	10	4.5	
	Nodule (1%). .	+	+++	++++	12	11.9	
	Dialysed nodule.	-	++	++	5	3.4	
	Dialysed filtrate.	+	++	++	5	4.4	
Bean.	Control. . . .	++	++	++	6	1.3	
	Yeast.	++	++	++	9	4.1	
	Nodule (1%). .	++	+++	+++	11	9.7	
	Dialysed nodule.	-	++	++	6	4.3	
	Dialysed filtrate.	++	++	++	6	1.7	
Clover.	Control. . . .	+	++	++	5	2.3	
	Yeast.	++	++	++	9	6.6	
	Nodule (1%). .	++	++	+++	11	12.1	
	Dialysed nodule.	++	++	++	8	3.4	
	Dialysed filtrate.	++	++	++	6	3.0	

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

The procedure for the preparation of each extract is described below :

- A.) *Cold water extract*.— Five grams of nodules were taken directly and dialysed with 280 m.a. maximum and 26 m.a. minimum current.
- B.) *Hot water extract*.— Five grams of nodules was brought to boiling on the water bath with 500 cc. water and kept boiling for 30 min, filtered and dialysed with 95 m.a. maximum and 28 m.a. minimum current.
- C.) *Alcoholic extract*.— Five grams of nodules were extracted with alcohol until no more color comes out by SOXHLET method and dialysed with 38 m.a. maximum and 16 m.a. minimum current.
- D.) *Alcohol and hot water extract*.— The residue from the alcoholic extract was subjected to hot water extraction, and dialysed with 55 m.a. maximum and 18 m.a. minimum current.
- F.) *Yeast extract*.— The yeast extract was dialysed in order to obtain the comparative results.

These extracts were concentrated on the water bath and a portion corresponding to 1 percent of the original sample was added to the medium and the tests were carried out but in the case of yeast extract, 10 percent portion was used. The results are presented in Table 2.

(See Table 2 on next page.)

As noted in Table 2, the best growth was obtained where the nodules were added, followed by the yeast extract and all the others which came from the anodic chamber were not effective. Those came from the middle chamber were about intermediate while in the case of yeast, the substance from the middle chamber was the best. Morphologically the rod and short rod were prevalent and the bacteroids were observed in a few instances where the anodic substance was added.

The results obtained with Genge nodule bacteria, Strain B are given in Table 3.

(See Table 3 on page 522.)

The best growth was obtained with the original solution of yeast extract and nodules, followed by alcoholic residue while the hot water and alcoholic-hot water extract (anodic) gave bad growth, and the others were just about the same with Strain A. The yeast extract in the middle chamber was effective. Morphologically no marked change was observed.

The results obtained with Genge nodule bacteria, Strain C are noted in Table 4.

(See Table 4 on page 523.)

As the data in Table 4 indicate, the best growth was obtained by the original solution of yeast and nodule extracts, and the alcoholic and hot water extracts (anodic) were the worst. More of the accessory substance was found in the anodic chamber and less in the cathodic. In other respects, the results were similar to those obtained in the previous experiment. A majority of cells were rod and few was coccic.

Table 2.
Influence of Different Portion of Dialysed Nodules on the Growth
of Genge Nodule Bacteria, Strain A.

Agents of extraction.	Substance added.	Rate of growth by days.				Cells on 7th day.	
		2	4	7	Sum of +.	Form.	Size. (μ)
Control.		++	++	++	6	Short rod.	0.3×0.4 - 0.4×0.8
Nodules.		+	####	####	13	"	0.2×0.5 - 0.3×1.0
Cold water.	Cathodic. .	-	###	###	8	"	0.2×0.4 - 0.5×0.8
	Anodic. . .	-	+	+	2	"	0.3×0.5 - 0.6×1.0
	Undialysed.	+	###	###	9	Rod.	0.2×0.5 - 0.3×1.2
	Residue. . .	+	##	##	7	"	0.2×0.5 - 0.3×1.2
Hot water.	Cathodic. .	+	##	###	9	Short rod.	0.2×0.5 - 0.4×0.8
	Anodic. . .	-	+	+	2	"	0.3×0.5 - 0.6×1.0
	Undialysed.	+	##	###	8	Rod.	0.2×0.5 - 0.4×1.2
	Residue. . .	+	##	##	7	Short rod.	0.3×0.5 - 0.4×0.8
Alcohol.	Cathodic. .	+	##	###	8	"	0.2×0.4 - 0.3×0.7
	Anodic. . .	+	+	+	3	Rod.	0.3×0.5 - 0.6×1.5
	Undialysed.	+	++	++	5	"	0.3×0.5 - 0.4×1.3
	Residue. . .	+	##	###	9	Short rod.	0.2×0.5 - 0.6×1.0
Alcoholic residue and hot water.	Cathodic. .	+	++	##	6	Rod.	0.3×0.5 - 0.4×1.2
	Anodic. . .	+	+	+	3	"	0.3×0.5 - 0.5×1.2
	Undialysed.	+	+	++	4	Short rod.	0.3×0.5 - 0.5×0.8
	Residue. . .	+	##	##	7	Rod.	0.2×0.5 - 0.6×1.2
Alcoholic extract and hot water extract.	Cathodic. .	-	##	###	7	"	0.3×0.5 - 0.5×1.2
	Anodic. . .	-	+	+	2	"	0.2×0.5 - 0.4×2.0
	Undialysed.	-	###	###	9	"	0.3×0.5 - 0.5×1.2
	Residue. . .	++	++	++	6	Short rod.	0.2×0.4 - 0.4×0.7
Yeast.	Cathodic. .	+	++	++	5	"	0.2×0.5 - 0.5×0.8
	Anodic. . .	++	++	++	6	Rod.	0.3×0.5 - 0.6×1.2
	Undialysed.	+	###	###	10	Short rod.	0.3×0.5 - 0.6×1.0
	Original solution.	++	###	###	12	"	0.2×0.3 - 0.3×0.7

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

Table 3.
Influence of Different Portion of Dialysed Nodules on the Growth
of Genge Nodule Bacteria, Strain B.

Agents of extraction.	Substance added.	Rate of growth by days.				Cells on 7th day.	
		2	4	7	Sum of +.	Form.	Size. (μ)
Control.		+	++	++	5	Rod.	0.3×0.5-0.8×1.5
Nodules.		###	####	####	14	"	0.2×0.5-0.6×1.2
Cold water.	Cathodic. .	++	####	####	12	"	0.3×0.5-0.5×1.2
	Anodic. . .	+	+	+	3	"	0.3×0.5-0.6×1.2
	Undialysed.	++	###	###	10	"	0.3×0.5-0.5×1.2
	Residue. . .	###	###	###	12	Short rod.	0.2×0.4-0.3×0.8
Hot water.	Cathodic. .	++	##	###	9	Rod.	0.3×0.5-0.5×1.5
	Anodic. . .	-	+	+	2	"	0.3×0.5-0.4×1.2
	Undialysed.	##	###	###	11	"	0.2×0.5-0.6×1.5
	Residue. . .	++	++	##	7	"	0.3×0.5-0.4×1.2
Alcohol.	Cathodic. .	++	###	###	10	Short rod.	0.3×0.5-0.5×0.8
	Anodic. . .	+	+	+	3	Rod.	0.3×0.5-0.5×1.2
	Undialysed.	+	++	##	6	"	"
	Residue. . .	##	####	####	13	"	"
Alcoholic residue and hot water.	Cathodic. .	+	++	##	6	"	0.2×0.5-0.4×1.2
	Anodic. . .	+	+	+	3	"	0.3×0.5-0.5×1.2
	Undialysed.	+	++	##	6	"	0.2×0.4-0.5×1.2
	Residue. . .	++	##	###	9	"	0.2×0.5-0.6×1.5
Alcoholic extract and hot water extract.	Cathodic. .	+	###	###	9	"	0.2×0.5-0.4×1.2
	Anodic. . .	-	+	+	2	"	0.3×0.7-0.5×2.5
	Undialysed.	-	##	##	6	"	0.2×0.5-0.4×1.2
	Residue. . .	++	++	##	7	"	0.3×0.5-0.4×1.5
Yeast.	Cathodic. .	+	++	++	5	Short rod.	0.2×0.3-0.5×0.8
	Anodic. . .	+	+	++	4	"	0.2×0.5-0.4×1.0
	Undialysed.	##	###	###	11	Rod.	0.2×0.5-0.6×1.2
	Original solution.	###	####	####	14	"	0.2×0.5-0.5×1.2

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

Table 4.
Influence of Different Portion of Dialysed Nodules on the Growth
of Genge Nodule Bacteria, Strain C.

Agents of extraction.	Substance added.	Rate of growth by days.				Cells on 7th day.	
		2	4	7	Sum of +.	Form.	Size. (μ)
Control.		++	++	++	6	Short rod.	0.3×0.4-0.4×1.0
Nodules.		###	###	###	14	Rod.	0.3×0.5-0.5×1.2
Cold water.	Cathodic. .	-	##	###	7	"	0.2×0.5-0.6×1.2
	Anodic. . .	+	+	+	3	Short rod.	0.3×0.4-0.6×1.0
	Undialysed.	++	++	++	6	Rod.	0.2×0.5-0.6×1.2
	Residue. . .	##	##	##	9	"	"
Hot water.	Cathodic. .	##	###	###	12	"	0.3×0.5-0.6×1.2
	Anodic. . .	-	+	++	3	Short rod.	0.2×0.4-0.6×1.0
	Undialysed.	##	###	###	11	Rod.	0.3×0.5-0.6×1.2
	Residue. . .	##	##	##	9	"	0.2×0.4-0.3×1.2
Alcohol.	Cathodic. .	##	###	###	13	Short rod.	0.2×0.5-0.4×1.0
	Anodic. . .	+	+	+	3	"	0.2×0.5-0.6×1.0
	Undialysed.	##	##	##	10	Short rod-coccic.	0.3×0.4-0.5×0.8
	Residue. . .	##	##	##	9	Rod.	0.2×0.5-0.3×1.2
Alcoholic residue and hot water.	Cathodic. .	##	##	##	9	Short rod.	0.2×0.5-0.4×0.8
	Anodic. . .	+	+	+	3	Rod.	0.3×0.5-0.5×1.5
	Undialysed.	++	++	++	6	Short rod.	0.2×0.5-0.5×1.0
	Residue. . .	++	##	##	9	Rod.	0.3×0.5-0.6×1.5
Alcoholic extract and hot water extract.	Cathodic. .	++	###	###	12	Short rod.	0.3×0.5-0.5×0.7
	Anodic. . .	-	+	+	2	"	0.3×0.5-0.5×1.0
	Undialysed.	+	##	##	9	"	0.2×0.4-0.5×0.8
	Residue. . .	++	##	##	8	Rod-coccic.	0.3×0.4-0.5×1.2
Yeast.	Cathodic. .	+	+	+	3	Short rod.	0.3×0.4-0.4×0.6
	Anodic. . .	+	+	+	3	"	0.3×0.5-0.5×1.0
	Undialysed.	##	###	###	11	"	0.2×0.5-0.7×1.0
	Original solution.	##	##	##	14	"	0.2×0.5-0.3×1.0

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

The results obtained with the bean nodule bacteria are presented in Table 5.

Table 5.
Influence of Different Portion of Dialysed Nodules on the Growth
of Bean Nodule Bacteria.

Agents of extraction.	Substance added.	Rate of growth by days.				Cells on 7th day.	
		2	4	7	Sum of +.	Form.	Size. (μ)
Control.		++	++	++	6	Short rod.	$0.3 \times 0.5 - 0.5 \times 0.7$
Nodules.		###	####	####	16	Oval.	$0.4 \times 0.6 - 0.7 \times 1.0$
Cold water.	Cathodic. .	+	###	###	9	Short rod.	$0.3 \times 0.5 - 0.5 \times 0.8$
	Anodic. . .	-	##	##	6	"	"
	Undialysed.	##	##	##	11	Rod.	$0.3 \times 0.5 - 0.5 \times 2.0$
	Residue. . .	##	##	##	11	"	$0.3 \times 0.5 - 0.7 \times 1.2$
Hot water.	Cathodic. .	##	##	###	12	Short rod.	$0.3 \times 0.5 - 0.5 \times 0.8$
	Anodic. . .	+	++	++	5	"	$0.3 \times 0.5 - 0.5 \times 1.0$
	Undialysed.	++	##	##	9	Rod.	$0.3 \times 0.5 - 0.6 \times 1.2$
	Residue. . .	##	##	##	11	Short rod.	$0.3 \times 0.5 - 0.5 \times 1.0$
Alcohol.	Cathodic. .	##	##	##	11	Rod.	$0.2 \times 0.5 - 0.5 \times 1.2$
	Anodic. . . .	++	++	++	6	"	$0.3 \times 0.5 - 0.5 \times 1.5$
	Undialysed.	+	##	##	7	Short rod.	$0.2 \times 0.5 - 0.5 \times 0.8$
	Residue. . .	++	##	##	8	"	$0.2 \times 0.5 - 0.6 \times 1.0$
Alcoholic residue and hot water.	Cathodic. .	++	##	##	8	"	$0.2 \times 0.5 - 0.3 \times 0.8$
	Anodic. . .	++	++	++	6	Rod.	$0.3 \times 0.5 - 0.6 \times 1.5$
	Undialysed.	+	++	###	7	Short rod.	$0.2 \times 0.5 - 0.4 \times 0.8$
	Residue. . .	##	###	###	13	"	$0.3 \times 0.5 - 0.5 \times 1.0$
Alcoholic extract and hot water extract.	Cathodic. .	+	##	##	9	"	$0.3 \times 0.5 - 0.4 \times 0.8$
	Anodic. . . .	-	+	+	2	Rod.	$0.2 \times 0.5 - 0.6 \times 2.0$
	Undialysed.	-	##	##	8	Coccie-rod.	$0.2 \times 0.4 - 0.5 \times 0.7$
	Residue. . .	##	##	##	10	Short rod.	$0.2 \times 0.5 - 0.6 \times 0.8$
Yeast.	Cathodic. .	++	++	++	6	"	$0.2 \times 0.5 - 0.4 \times 1.0$
	Anodic. . . .	+	++	++	5	Rod.	$0.3 \times 0.5 - 0.6 \times 1.2$
	Undialysed.	++	##	##	10	"	$0.2 \times 0.5 - 0.6 \times 1.5$
	Original solution.	###	##	##	15	Short rod.	$0.2 \times 0.5 - 0.3 \times 1.0$

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

Table 5 indicates that the original nodule extract was most effective, followed by the yeast extract, and the alcoholic and hot water extracts (anodic) were least effective. A majority of cells were short rod or rod and some oval, and coccie cells were found.

The similar experiment was carried out with the clover nodule bacteria and the results are given in Table 6.

Table 6.
Influence of Different Portion of Dialysed Nodules on the Growth
of Clover Nodule Bacteria.

Agents of extraction.	Substance added.	Rate of growth by days.				Cells on 7th day.	
		2	4	5	Sum of +.	Form.	Size. (μ)
Control.		++	##	##	8	Rod.	$0.3 \times 0.5 - 0.5 \times 1.5$
Nodules.		###	###	###	14	"	$0.3 \times 0.5 - 0.5 \times 1.2$
Cold water.	Cathodic. .	##	###	###	13	"	$0.3 \times 0.5 - 0.5 \times 1.2$
	Anodic. . .	++	##	##	8	"	$0.3 \times 0.6 - 0.6 \times 1.5$
	Undialysed.	##	##	##	11	"	$0.4 \times 0.5 - 0.6 \times 1.5$
	Residue. . .	++	##	##	8	"	$0.3 \times 0.5 - 0.5 \times 1.2$
Hot water.	Cathodic. .	##	###	###	13	"	$0.3 \times 0.6 - 0.7 \times 1.2$
	Anodic. . .	+	++	##	7	Short rod.	$0.2 \times 0.5 - 0.4 \times 1.0$
	Undialysed.	++	##	##	9	Rod.	$0.3 \times 0.6 - 0.7 \times 1.2$
	Residue. . .	++	##	###	11	Short rod.	$0.2 \times 0.5 - 0.4 \times 1.0$
Alcohol.	Cathodic. .	###	###	###	14	"	$0.2 \times 0.5 - 0.6 \times 1.2$
	Anodic. . .	+	++	##	6	"	$0.3 \times 0.6 - 0.5 \times 1.5$
	Undialysed.	##	###	###	13	"	$0.3 \times 0.5 - 0.6 \times 1.3$
	Residue. . .	++	##	##	9	"	$0.3 \times 0.5 - 0.5 \times 1.0$
Alcoholic residue and hot water.	Cathodic. .	##	###	###	13	"	$0.2 \times 0.5 - 0.6 \times 1.0$
	Anodic. . .	+	++	##	6	"	"
	Undialysed.	+	++	##	6	Rod.	$0.2 \times 0.5 - 0.6 \times 1.5$
	Residue. . .	+	##	##	9	Short rod.	$0.2 \times 0.5 - 0.6 \times 1.0$
Alcoholic extract and hot water extract.	Cathodic. .	##	##	###	12	"	$0.4 \times 0.5 - 0.5 \times 1.0$
	Anodic. . .	-	++	++	4	Rod.	$0.2 \times 0.5 - 0.6 \times 2.5$
	Undialysed.	+	##	###	10	Short rod-oval.	$0.4 \times 0.5 - 0.6 \times 1.0$
	Residue. . .	++	##	###	11	Rod.	$0.3 \times 0.5 - 0.5 \times 1.8$
Yeast.	Cathodic. .	+	##	##	9	Short rod.	$0.3 \times 0.5 - 0.6 \times 1.0$
	Anodic. . .	++	##	##	10	Rod.	$0.2 \times 0.5 - 0.4 \times 1.2$
	Undialysed.	##	##	##	11	"	$0.2 \times 0.5 - 0.6 \times 1.5$
	Original solution.	##	###	###	14	"	$0.3 \times 0.5 - 0.5 \times 1.5$

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

Good growth was obtained with the original extracts of yeast and nodules, and also with the alcoholic (cathodic) extract, followed by the other extracts (cathodic). The growth was bad with the alcoholic and hot water extract (anodic).

Considering the foregoing results, the original yeast and nodule extracts were effective in all the cases as expected, and among the dialysed extracts, the anodic content was most effective, followed by the neutral and cathodic contents except the yeast extract of which the neutral content was most effective.

Determination of nitrogen and hydrogen ion concentrations:

The nitrogen contents in each portion of electrolysed material was determined by KJELDAHL method and the P_H value, by the quinhydrone electrode, and the results are noted in Table 7.

Table 7.
Nitrogen Contents and P_H Values of Dialysed Substance.

Agents of extraction.	Nature of dialysis.	Nitrogen contents.	P_H	Agents of extraction.	Nature of dialysis.	Nitrogen contents.	P_H
Cold water.	Cathodic.	(%) 0.02482	8.99	Alcohol residue + hot water.	Cathodic.	(%) 0.02115	8.51
	Anodic.	0.00592	2.52		Anodic.	0.00719	2.52
	Undialysed.	0.02672	3.39		Undialysed.	0.02190	3.35
Hot water.	Cathodic.	0.01918	8.36	Alcohol extract + hot water extract.	Cathodic.	0.01382	8.59
	Anodic.	0.00506	2.34		Anodic.	0.00338	2.40
	Undialysed.	0.02651	3.30		Undialysed.	0.01861	3.27
Alcohol.	Cathodic.	0.01015	8.01	Yeast.	Cathodic.	0.01015	8.36
	Anodic.	0.00169	3.03		Anodic.	0.00677	2.35
	Undialysed.	0.01139	3.70		Undialysed.	0.04258	3.20

As shown above, the largest amount of nitrogen was found in the middle chamber or the neutral portion and smallest in the cathodic. The cathodic was acid and the anodic was alkaline while the middle chamber was acid. Considering these results in conjunction with the foregoing experimental data, the amount of nitrogen has little to do in stimulating the growth of nodule bacteria since the anodic material which contained less nitrogen was more effective than that in the middle chamber where more nitrogen was found. On the other hand, the P_H value has a great influence over the growth but the difference of P_H values is not very marked when the culture medium is made up although the original extracts showed a marked difference.

Summary.

In this investigation, the electrical nature of the accessory substance by using the various extracts prepared from the bean nodules by cold and hot water,

alcohol and hot water together with the yeast extract. From the results obtained, the following summary may be made.

1.) By electro-dialysis, the accessory substance in the bean nodules was removed partly but not completely.

2.) In all the extracts, the accessory substance was chiefly found in the cathodic chamber and some in the middle while practically none in the anodic chamber.

3.) Morphologically a majority of cells were short rod and rod, and the bacteroides were comparatively few which were found more in the anodic chamber.

4.) Electrically the accessory components in the nodules and yeasts seems to be different. The yeast extract was very inactive.

5.) No relation between the accessory substance and the nitrogen content was found although the hydrogen ion concentration of the original solution seemed to have some influence.

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Literature.

- 1.) ITANO, A. and A. MATSUURA, Nōgaku Kenkyū. (In manuscript.)
 - 2.) Ibid.
 - 3.) Ibid. 14:432, 1930.
 - 4.) KONDO, K., Japanese Soc. for Advancement of Science, 6:284, 1930.
 - 5.) ITANO, A. and A. MATSUURA, Nōgaku Kenkyū, 22:218, 1934.
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