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Some Aspects of the Mechanism of Non-Symbiotic Fixation of Atmospheric Nitrogen.

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Some Aspects of the Mechanism of Non-Symbiotic Fixation of Atmospheric Nitrogen.

PREVIOUS studies on the economy of carbon during fixation of atmospheric nitrogen by *Azotobacter*, particularly by Stoklasa¹ and by Ranganathan and Norris² would suggest that the nitrogen fixers derive their organic nutrition chiefly from carbohydrates, though small quantities may be fixed in presence of other organic substances as well.

Our studies with the mixed flora of the soil showed that glucose which was provided as the organic nutrient was completely decomposed in the course of the first four days, being mostly converted into gases. Of the residual organic matter, 44.1 per cent. was accounted by micro-organisms (living as well as dead), 34.0 per cent. by organic acids (chiefly lactic, acetic, propionic and butyric) and the rest in some (yet unidentified) water soluble form. During this period only about a third of the usual quantity of nitrogen was fixed, and of this, the major part was present in water soluble form. In the course of the next four days a large part of the organic acids was lost, accompanied by corresponding increase in mucilage. There was also rapid fixation of atmospheric nitrogen, the C-N ratio of the organisms changing from 62.1 to 20.6. Between the 8th and the 12th days, there was very little change in the other constitu-

ents, but there was further fixation of nitrogen. After the 12th day, there was slight loss of organic carbon, but there was no appreciable fixation of nitrogen.

TABLE II.
Nitrogen Fixed and C-N Ratio.

Time in days	Nitrogen fixed in milligrams in 50 c.c. medium		C/N	
	Total	In bacterial tissue	In the whole medium	In bacterial tissue
0	Nil	Nil
2	0.78	0.39	143.2	43.9
4	1.26	0.39	43.7	62.1
8	2.42	1.93	22.1	20.6
12	3.07	2.90	18.0	13.8
16	3.15	2.95	16.0	13.4

TABLE III.
Distribution of Organic Acids (so far identified).

Time in days	Organic acids in mg. of carbon (in 50 c.c. medium)					Total acids
	Non-volatile	Volatile				
	Lactic	Acetic	Propionic	Butyric	Total	
2	6.1	2.7	0.6	3.8	7.1	13.2
4	12.8	3.1	0.4	3.5	7.0	19.8
8	Nil	5.8
12	Nil	4.8

TABLE I.
Distribution of Organic Carbon.

Time in days	Carbon in mg. (as present in 50 c.c. of medium)				
	Total organic carbon	Sugar	Organic acids (so far identified)	Microbial tissue	Unidentified
0	173.7	170.4	Nil	Nil	Nil
2	111.7	83.6	13.2	13.6	Nil
4	55.0	Nil	19.8	24.2	7.7
8	53.4	Nil	5.8	39.8	4.5
12	55.5(?)	Nil	4.8	39.9	7.5(?)
16	50.5	Nil	..	39.4	..

Carbonate carbon (including dissolved CO₂) was estimated at each stage, but has not been included. The soil used for inoculation contained 3.3 mg. of carbon,

It would be seen from the above that (a) the carbohydrate contributes only partly to the fixation; (b) organic acids are mostly utilised for the growth of the organisms and fixation of nitrogen; and (c) the production of mucilage and nitrogen fixation, though related, are not directly proportional to each other.

That the immediate products of decomposition of sugar are utilised in the fixation is further proved by the following results (Table IV):—

TABLE IV.

Time in days	Nitrogen fixed in mg. (in 50 c.c. of medium)	
	Residue sterilised and freshly inoculated	Unsterilised (control)
4	0.25	2.10
8	1.62	2.62

Further work is in progress to determine (a) the manner in which organic acids or their calcium salts assist in fixation, and (b) the nature of the water-soluble nitrogen

formed in the early stages and its relation to the subsequent fixation. The studies are also being extended to pure strains of nitrogen fixers.

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¹ Stoklasa, *Zentralbl. Bakt.* II, 1908, 21, 408.

² Ranganathan and Norris, *J. Indian Inst. Sci.*, 1927, 10A, 79.

