

of sodium acetylacetone and allowed to stand for a week. The new sodium derivative was dissolved in ice-water and decomposed with cold dil. acid. A vivid yellow oil separated and slowly hardened on standing. Only about a gram of the product was obtained. It crystallized from benzene mixture in shining yellow plates, m. p. 124-125°.

Calc. for  $C_{13}H_{15}O_2NS$ : N, 5.6. Found: 6.1.

**Thio-diaceto-acetyl-*o*-toluide**,  $CH_3COCH(CSNHC_6H_4CH_3)COCH_3$ .— Five g. of acetylacetone was converted into the sodium derivative and kept for a week with the equivalent of *o*-tolyl isothiocyanate mixed with ether. The mixture treated in the customary manner, gave 3 g. of crude material. This was purified by use of benzene-petroleum ether. Pale yellow irregular plates were obtained, m. p. 126.5-128°.

Calc. for  $C_{13}H_{15}O_2NS$ : N, 5.6. Found: 6.1.

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[CONTRIBUTION FROM THE OFFICE OF PLANT PHYSIOLOGICAL AND FERMENTATION INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.]

## THE MOSAIC DISEASE OF SPINACH AS CHARACTERIZED BY ITS NITROGEN CONSTITUENTS.<sup>1</sup>

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### Introduction.

Spinach belongs to leafy vegetables which are essentially different from what may be classed as seeds, roots, and tubers. While the latter<sup>2</sup> representing storage organs contain chiefly reserve proteins, carbohydrates and fats, and but very little active protoplasm, the former<sup>3</sup> are made up of a large quantity of functioning cells combined with reserve food material. Whereas roots,<sup>4</sup> seeds and tubers are deficient in certain inorganic elements and in the dietary essential, fat soluble vitamine A, containing at the same time proteins of low biological value, leaves are comparatively rich in inorganic elements, especially in calcium and sodium, as well as in the fat-soluble vitamine A. This is especially true of spinach<sup>5</sup> (*Spinacia oleracea*), which is comparatively rich both in the fat-soluble vitamine A and in the water-soluble vitamine B, being twice as efficient as whole wheat, soy beans, dried eggs or milk solids. These important nutritive qualities of spinach make it desirable to ascertain the nature of the mosaic disease of spinach with the object in view of finding a proper remedy for the disease.

<sup>1</sup> Presented before the Division of Biological Chemistry at the St. Louis meeting of the American Chemical Society, April 12-17, 1920.

<sup>2</sup> McCollum, Simmonds and Parsons, *J. Biol. Chem.*, 38, 115 (1919).

<sup>3</sup> McCollum, Simmonds and Pitz, *ibid.*, 30, 19 (1917).

<sup>4</sup> *Loc. cit.*

<sup>5</sup> Osborne and Mendel, *ibid.*, 37, 190 (1919).

Briefly stated, the mosaic disease of spinach, widely known as "spinach-blight," is characterized by a few well-defined symptoms. While the leaves of normal spinach plants have a uniform deep-green color, those of blighted plants show a rather yellowish green color, especially between the veins being at the same time mottled and malformed. Whereas the roots of healthy plants are well developed, those of diseased plants are characterized by their shrunken appearance and a loss of lateral rootlets. Generally speaking, the blighted plants show considerable dwarfing, so that the effect of the disease<sup>1</sup> may be said to be a material reduction of the crop (up to 20%) and a lowering of its quality.

In previous papers it was demonstrated that the disease is not due to the inability of the blighted plants to produce carbohydrates,<sup>2</sup> as is evident from the fact that leaves of diseased spinach have even a larger proportion of carbohydrates than those of normal plants, there being no marked difference in the diastatic activities of blighted and healthy plants. It was further shown that spinach plants affected with the mosaic disease have a lower ash<sup>3</sup> content (in the tops) but a higher oxidase<sup>4</sup> content than do normal plants. While the above data are of considerable value and interest, they failed to reveal the nature of the spinach disease. It was with the hope of attaining this object that a study of the nitrogen metabolism<sup>5</sup> in the spinach plant was undertaken in 1915. This study has led, among other things, to the assumption that denitrification takes place in the diseased plants, it being based largely on the facts that the diseased plants have been found to contain less total and acid amide nitrogen but more ammoniacal nitrogen than the normal plants. The results, however, may have been incidental to the particular spinach samples of that season. Hence, it seemed important, if not imperative, to corroborate those results by observations on spinach samples taken from various other fields and at other seasons. This was also prompted by the consideration that the disease in question assumes greater significance in view of the fact that a disease similar to or identical with spinach blight has been found to affect several other plants of vast economic importance.

Now if denitrification takes place in diseased spinach we should naturally expect less nitrates in blighted plants than in normal, while nitrites, if present at all, would be expected in the former rather than in the latter. Moreover, in the case of proteins hydrolyzed and separated according to Hausmann's method, the phosphotungstic acid precipitate and the filtrate from it consist of diamino and monoamino acids, respectively.

<sup>1</sup> A complete description of the disease is given by McClintock and Smith, *J. Agr. Research*, 14, 2 (1918).

<sup>2</sup> True and Hawkins, *ibid.*, 15, 381 (1918).

<sup>3</sup> True, Black and Kelly, *ibid.*, 15, 372 (1918).

<sup>4</sup> *Ibid.*, 15, 377 (1918).

<sup>5</sup> Jodidi, Kellogg and True, *ibid.*, 15, 385 (1918).

This, however, may not necessarily hold good for spinach, since plants are known to contain, in addition to amino acids and acid amides, also nuclein bases, purin bodies, alkaloids, etc. For this reason it was of considerable moment to find out just what proportion of the basic and non-basic nitrogen as represented by the phosphotungstic acid precipitate and its filtrate is actually made up of diamino and monoamino acids, respectively. This work, too, suggested itself for the reason that while amino acids are known to have nutritive value, it is doubtful whether other nitrogenous compounds, such as acid amides, ammonia, etc., play any role in nutrition.

### Experimental.

The spinach samples, both healthy and diseased, used in this work were taken in April, 1918, from beds on the Childreth farm at Lenox, Va.<sup>1</sup> They were separately dried first at room temperature for several days, to be then subjected to the temperature of about 50° for 1 to 2 days in an electric drying oven in which slight vacuum was maintained. The dried materials were then powdered, passed through a 40-mesh sieve and put into sealed jars, ready for use.

**Methods.**—The *total nitrogen* of the spinach materials was estimated according to the Gunning modification of Kjeldahl's method.

The *nitric nitrogen* was determined as follows. A definite amount of the spinach material was repeatedly extracted with alcohol (85%), the combined extracts made alkaline with milk of lime and evaporated to dryness at low temperature. The residue was taken up with hot water, treated with lead acetate solution, and finally made up to 1000 cc. Of the perfectly clear filtrate aliquot portions, usually 400 cc., were concentrated to about 50 cc., and their nitric acid content determined according to Schulze-Tiemann's<sup>2</sup> method.

The estimation of *ammonia* was carried out according to Grafe's<sup>3</sup> method, whereby the finely powdered materials are treated with a saturated solution of sodium carbonate mixed with sodium chloride solution, and the ammonia thus set free is distilled off *in vacuo* at a temperature ranging from 25 to 37°. Alcohol is usually added to prevent foaming.

As to nitrous acid the spinach materials were examined only qualitatively.

*Protein nitrogen* was determined according to Stutzer's<sup>4</sup> method, whereby the protein is precipitated essentially by means of a glycerol-copper

<sup>1</sup> The authors wish to express their appreciation to Prof. T. C. Johnson, Director, Va. Truck Expt. Station, and to Mr. J. B. Norton, Office of Cotton, Truck and Forage-Crop Disease Investigations, for valuable assistance in securing the spinach samples.

<sup>2</sup> Abderhalden's "Handbuch biochem. Arbeitsmeth.," 6, 312 (1912).

<sup>3</sup> Z. *physiol. Chem.*, 48, 300 (1906).

<sup>4</sup> J. *Landw.*, 28, 105 (1880); 29, 475 (1881); Z. *physiol. Chem.*, 6, 573 (1882).

hydroxide suspension, the precipitate obtained being oxidized according to the Kjeldahl method.

The separation of the *non-protein* nitrogen into the various groups was carried out according to Hausmann's method whereby one g. of magnesium oxide was used for distilling off the acid amide<sup>1</sup> nitrogen. Other methods used in this work will be described subsequently.

The results secured are summarized in the following tables:

TABLE I.—PERCENTAGE OF NITROGEN IN THE OVEN-DRIED SPINACH.

Healthy spinach material.	Nitrogen found. %.	Blighted spinach material.	Nitrogen found. %.	Difference in nitrogen content. %.
Leaves (Sample A)	4.98	Leaves (Sample A)	3.89	—21.89
Leaves (Sample A)	4.98	Leaves (Sample A)	3.83	—23.09
Leaves (Sample A)	5.03	Leaves (Sample A)	3.92	—22.06
Leaves (Sample B)	4.90	Leaves (Sample B)	3.79	—22.65
Leaves (Sample B)	4.82	Leaves (Sample B)	3.80	—21.16
Leaves (Sample B)	4.90	Leaves (Sample B)	3.80	—22.45
Roots	3.90	Roots	3.95	+1.28
Roots	3.90	Roots	3.92	+0.51
Roots	3.94	Roots	3.88	—1.52
....			3.89	.....

### Discussion.

By reference to Table I it will be seen that, under normal conditions, the proportion of nitrogen in the leaves is considerably higher (up to 25%) than in the roots, while the nitrogen content of blighted leaves and roots is about the same. Further, it will be noticed that the nitrogen content of the leaves, which constitute the bulk of the plant, is very much lower in the diseased plants than in the healthy ones, the difference ranging from 21.16 to 23.09%, while the difference in the nitrogen content of the diseased and healthy roots is so small as to be negligible. It may be mentioned here that similar results were obtained with spinach samples of the season 1915-1916,<sup>2</sup> the diseased leaves and the plants as a whole showing in *all instances* a considerably lower nitrogen content than the normal ones, so that the *lower nitrogen content of the diseased tissues may safely be considered as one of the striking characteristics of the mosaic disease of spinach.*

Examination of Table II reveals the fact that the leaves of the healthy spinach have normally a somewhat higher nitric nitrogen content than the roots, the reverse being true of the blighted spinach. The chief difference, however, lies in the tops, the healthy leaves being very much richer in nitric nitrogen (up to 50%) than the diseased, while the difference in the nitrate content of the healthy and blighted roots is but slight. Inasmuch as the distinctions noticed hold good for *all samples and seasons*

<sup>1</sup> Jodidi and Moulton, *THIS JOURNAL*, 41, 1526 (1919).

<sup>2</sup> Jodidi, Kellogg and True, *J. Agr. Research*, 15, 390 (1918).

TABLE II.—PERCENTAGE OF NITRIC NITROGEN IN THE SPINACH (SEASON 1918).

Healthy spinach material.	Nitric nitrogen.			Diseased spinach material.	Nitric nitrogen.		
	Soluble nitrogen. %.	Oven-dried spinach. %.	Total nitrogen. %.		Soluble nitrogen. %.	Oven-dried spinach. %.	Total nitrogen. %.
Leaves (Sample 1)	6.87	0.17	3.42	Leaves (Sample 1)	4.65	0.08	2.20
Leaves (Sample 1)	6.89	0.17	3.43	Leaves (Sample 1)	4.65	0.08	2.20
Leaves (Sample 2)	7.69	0.19	3.83	Leaves (Sample 2)	5.97	0.11	2.82
Leaves (Sample 2)	8.07	0.19	4.02	Leaves (Sample 2)	5.84	0.11	2.76
Roots	6.30	0.13	3.30	Roots	....	0.12	3.01
				Roots	....	0.12	3.02

PERCENTAGE OF NITRIC NITROGEN IN THE SPINACH (SEASONS 1915 AND 1916).<sup>a</sup>

Leaves	6.62	0.17	3.36	Leaves	2.64	0.06	1.39
Leaves	6.86	0.18	3.48	Leaves	2.62	0.06	1.38
Leaves	6.80	0.18	3.45	Entire plant	3.06	0.06	1.60
Entire plant	7.41	0.19	3.92	Entire plant	3.02	0.06	1.58
Entire plant	7.31	0.19	3.87	Entire plant	2.96	0.05	1.55

<sup>a</sup> It goes without saying that the nitric nitrogen estimations of the spinach materials for the seasons 1915 and 1916 have not been published yet.

examined the conclusion seems to be justified that a *lower nitrate content of the blighted tissues is another striking characteristic of the mosaic disease of spinach.*

TABLE III.—PROPORTION OF NITROUS AND AMMONIACAL NITROGEN IN THE SPINACH.

Healthy spinach material.	Nitrites. %.	Ammoniacal nitrogen.			Diseased spinach material.	Nitrites. %.	Ammoniacal nitrogen.		
		Soluble nitrogen. %.	Oven-dried spinach. %.	Total nitrogen. %.			Soluble nitrogen. %.	Oven-dried spinach. %.	Total nitrogen. %.
Leaves	0.0	3.80	0.092	1.89	Leaves	Present	5.40	0.097	2.55
Leaves	0.0	3.80	0.092	1.89	Leaves	Present	5.40	0.097	2.55
Leaves	0.0	4.02	0.097	2.00					
Roots	0.0	5.12	0.105	2.68	Roots		5.80	0.094	2.41
Roots	0.0	5.04	0.103	2.64	Roots		5.80	0.094	2.41

Inspection of Table III shows that the proportion of ammoniacal nitrogen in the diseased leaves is higher than in the healthy, the difference in the ammonia content of the roots of diseased and normal plants being insignificant, and while nitrites are present in diseased tissues none could be detected in normal ones. Hence, *higher ammonia content and presence of nitrites may be said also to be characteristic of spinach blight.*

The importance of proteins as carriers of life in the plant organism and their significance in nutrition suggested the estimation of protein nitrogen in the various spinach tissues. A glance at Table IV shows that in the normal plants the proportion of protein and protein nitrogen is higher in the leaves than in the roots when calculated on the oven-dried

TABLE IV.—PROPORTION OF PROTEIN IN THE SPINACH.

	Protein nitrogen.			Corresponding protein.			Protein nitrogen.			Corresponding protein.	
	Fresh spin. ach. %.	Oven-dried spin. ach. %.	Total nitro. gen. %.	Fresh spin. ach. %.	Oven-dried spin. ach. %.		Diseased spinach material.	Fresh spin. ach. %.	Oven-dried spin. ach. %.	Total nitro. gen. %.	Fresh spin. ach. %.
Healthy spinach material.											
Leaves	0.38	2.72	55.75	2.38	17.00	Leaves	0.44	2.32	61.01	2.75	14.50
Leaves	0.38	2.66	54.58	2.38	16.63	Leaves	0.44	2.30	60.52	2.75	14.38
Leaves	0.38	2.72	55.86	2.38	17.00	Leaves	0.45	2.38	62.51	2.81	14.88
Roots	0.40	2.09	53.54	2.50	13.06	Roots	....	2.36	60.28	....	14.75
Roots	0.40	2.09	53.38	2.50	13.06	Roots	....	2.32	59.31	....	14.50
Roots	0.39	2.06	52.66	2.44	12.88	Roots	....	2.32	59.22	....	14.50

spinach and its total nitrogen, there being practically no difference in the corresponding diseased tissues. It further will be noticed that the percentage of protein and protein nitrogen is greater in the diseased tissues than in the healthy when referred to the oven-dried materials and their total nitrogen content, the only exception being the leaves which in health show a higher protein content than in disease, when calculated to the oven-dried spinach. All of which goes to show that the diseased plants are fully capable of building up proteins out of their nitrogen.

In order to establish, if possible, features characteristic of blighted spinach other than those already reported, the following experiments were carried out. Forty g. portions of spinach were repeatedly extracted with boiling hot, ammonia-free water, the combined extracts acidified with acetic acid, boiled for a few minutes, filtered, cooled and made up to 1000 cc. Of this solution representing the non-protein nitrogen, 3 portions of 25 cc. each were analyzed by the Kjeldahl method to ascertain the quantity of nitrogen present. Now 250 cc. portions of this solution were treated with enough conc. hydrochloric acid to make a 20% acid, kept boiling under reflux and finally separated into the various nitrogenous groups, according to Hausmann's method, as outlined in a previous<sup>1</sup> paper.

Inasmuch as, by hydrolysis, peptide linkings ( $\text{NH}_2\text{R}-\text{CO}-\text{NH}-\text{R}'$ .  $\text{COOH}$ ) are split asunder with the formation of free amino acids ( $\text{NH}_2\text{R}$ .  $\text{COOH} + \text{NH}_2\text{R}'\text{COOH}$ ), the peptide nitrogen was ascertained by estimating the formol-titrable nitrogen in the aqueous spinach extract and subtracting this result from the formol-titrable nitrogen found in the previously hydrolyzed spinach extract. In each case coloring matter, phosphoric acid and carbon dioxide were removed from the solutions prior to their formol-titration. References concerning details<sup>2</sup> of the method as applied here as well as its limitations will be found elsewhere.<sup>3</sup> The results obtained are presented in Table V.

<sup>1</sup> Jodidi and Moulton, *THIS JOURNAL*, 41, 1526 (1919).

<sup>2</sup> S. L. Jodidi, *ibid.*, 33, 1236 (1911); 34, 97 (1912).

<sup>3</sup> S. L. Jodidi, *ibid.*, 40, 1031 (1918).

TABLE V.—DISTRIBUTION OF THE WATER-SOLUBLE NITROGEN IN SPINACH.  
Data Expressed in Percentage of the Total Nitrogen of the Spinach  
Materials.

Spinach material.	Acid amide nitro-gen. %.	Humin nitro-gen. %.	Basic nitro-gen. %.	Non-basic nitro-gen. %.	Peptide nitro-gen. %.	Spinach material.	Acid amide nitro-gen. %.	Humin nitro-gen. %.	Basic nitro-gen. %.	Non-basic nitro-gen. %.	Peptide nitro-gen. %.
Healthy leaves	10.12	3.05	11.10	13.98	6.25	Diseased leaves	7.33	3.65	8.24	11.12	12.19
	10.11	3.02	11.11	14.91	5.34		7.31	4.31	9.31	12.62	8.98
	10.43	2.44	...	...	5.40		7.58	...	9.61	...	...
	...	...	...	...	6.10		...	...	...	...	...
Healthy roots	10.64	2.91	9.83	...	...	Diseased roots	7.44	...	8.05	...	...
	10.78	3.10	9.31	...	...		7.51	2.40	8.13	...	...
	10.83	3.01	9.23	...	...		7.72	3.01	8.36	...	...

Data Expressed in Percentage of the Soluble Nitrogen of the Spinach  
Materials.

Healthy leaves	21.20	6.34	23.10	26.15	12.54	Diseased leaves	15.48	7.71	17.41	23.56	25.79
	21.17	6.28	23.12	28.02	10.72		15.42	9.11	19.67	26.76	18.99
	21.85	5.08	...	...	10.84		16.00	...	20.19	...	...
	...	...	...	...	12.24		...	...	...	...	...
Healthy roots	20.32	5.56	18.77	...	...	Diseased roots	18.91	...	20.20	...	...
	20.58	5.91	17.78	...	...		19.10	6.02	20.40	...	...
	20.68	5.74	17.63	...	...		19.61	7.55	20.97	...	...

Data Expressed in Percentage of the Oven-Dried Spinach Materials.

Healthy leaves	0.498	0.15	0.54	0.68	0.30	Diseased leaves	0.283	0.14	0.31	0.43	0.46
	0.488	0.15	0.54	0.73	0.26		0.273	0.16	0.35	0.50	0.34
	0.513	0.12	...	...	0.26		0.293	...	0.37	...	...
	...	...	...	...	0.30		...	...	...	...	...
Healthy roots	0.415	0.11	0.38	...	...	Diseased roots	0.296	...	0.31	...	...
	0.417	0.12	0.36	...	...		0.296	0.09	0.32	...	...
	0.427	0.12	0.36	...	...		0.306	0.12	0.33	...	...

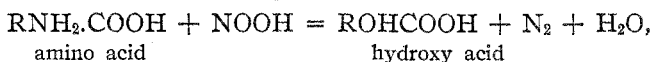
The proportion of diamino and monoamino nitrogen contained in the basic and non-basic nitrogen, respectively, was determined as follows. The phosphotungstic acid precipitate (basic nitrogen) and the filtrate from it (non-basic nitrogen) were separately treated with barium hydroxide whose excess was removed with carbon dioxide.

Filtrate and washings from the phosphotungstate, sulfate and carbonate of barium were evaporated on the water bath and finally made up to 100 cc., of which 2 portions of 20 cc. each were oxidized according to the Kjeldahl method to ascertain the quantity of nitrogen present. The remaining solution was freed from phosphoric acid and carbon dioxide as usual and formol-titrated. By this method it was ascertained in the case of

normal spinach leaves that, on an average, 41.97% of the basic nitrogen consists of diamino nitrogen, while 38.62% of the non-basic nitrogen is made up of monoamino nitrogen. In like manner it was found that, with diseased spinach leaves, the diamino nitrogen constitutes, on an average, 45.32% of the total basic nitrogen, while the monoamino nitrogen constitutes 39.75% of the total non-basic nitrogen.

### Interpretation of Results.

By referring to Tables V and IV it is readily seen that the diseased tissues, especially leaves, have a smaller proportion of acid amide, basic and non-basic nitrogen, but a larger proportion of peptide and protein nitrogen than the corresponding normal tissues. It is because of these findings, together with the differences in total, nitrate, nitrite, and ammoniacal nitrogen content already reported, that we logically and forcibly come to the conclusion that the pathological condition is brought about by the process of denitrification which takes place in the spinach tissues. This process, as is well known, consists in the reduction of nitrates to nitrites and partly also to ammonia. For this reason the diseased tissues contain less nitrate but more ammoniacal nitrogen than the normal, nitrites being present in the blighted tissues only (Tables II and III). Inasmuch as nitrous acid reacts on amino acids with elimination of the amino group according to the equation



we should expect less diamino (basic) and monoamino (non-basic) nitrogen in the diseased plants than in the normal ones, which is actually the case (Table V). It is true that the amido group in some of the acid amides (asparagine) does not react chemically on nitrous acid, at least not quantitatively.<sup>1</sup> However, under biological conditions (existing, *e. g.*, in the soil) the amido group in asparagine can be split off as easily as the amino group in amino acids (aspartic acid, etc.), as was demonstrated by one<sup>2</sup> of us. It was even shown that the amino group in acid amides which do not occur naturally, as in acetamide and propionamide,<sup>3</sup> can be split off with ease under the conditions mentioned. These considerations, then, give a satisfactory explanation as to why the proportion of acid amide nitrogen is smaller in the blighted tissues than in the normal ones. Loss of elementary nitrogen by the amino acids, acid amides and nitrates, as well as loss of ammonia as such fully accounts for the fact that the diseased tissues have a smaller percentage of total nitrogen. It is, then, not to be wondered at that loss of the very important plant food, nitrogen,

<sup>1</sup> D. D. Van Slyke, *J. Biol. Chem.*, **9**, 196 (1911).

<sup>2</sup> Jodidi, Kellogg and Snyder, *Iowa Agr. Expt. Sta. Research Bull.*, **9**, 350 (1912); S. L. Jodidi, *Eighth Intern. Congr. Appl. Chem.*, **26**, 129 (1912).

<sup>3</sup> S. L. Jodidi, *J. Frank. Inst.*, **175**, 250, 255 (1913).



by the spinach plants should lead to troubles chief among which is their dwarfing.

The proteins and polypeptides of the spinach tissues do not seem to have been attacked by the nitrous acid, probably for the reason that they are less soluble than the amino acids and acid amides and for the further reason that their very much larger molecules with but one amino group (at one end of the molecule) offer fewer points of attack than the smaller molecules of amino acids, each of which has one or two amino groups. The higher percentage of peptide and protein nitrogen in the diseased plants (calculated to the total nitrogen) is easily explained by the fact that here they are related to a *smaller* quantity of nitrogen, total and soluble, than in the normal plants where none of the nitrogen was lost through denitrification.

The question further arises as to why some of the attacked plants die altogether. The following contemplation seems to give a satisfactory answer. It is not out of the question that occasionally, under favorable conditions, the nitrous acid may attack proteins present in the spinach plant by reacting either on the amino group at the end of the protein molecule, or perhaps on some of the numerous CO-NH groups present. From the consideration, however, that proteins constitute an integral part of protoplasm, the carrier of life in the plant, it logically follows that a change in the chemical nature of the proteins, and hence of the protoplasm, must necessarily be fatal to the plant. This view-point is also corroborated by Loew's<sup>1</sup> theory according to which a substance which can act upon amino groups is a poison, and this is especially true of nitrous acid.

#### Summary.

The data at hand seem to warrant the following conclusions.

1. Spinach plants, especially their tops, affected with mosaic disease, have a smaller percentage of total nitrate, acid amide, mono and diamino nitrogen, but a somewhat larger percentage of ammonia than normal plants, nitrous acid being present in diseased plants only.

2. This is due to the fact that denitrification takes place whereby nitrates are reduced to nitrites which, reacting on the various nitrogenous compounds present in the spinach, bring about elimination of nitrogen in a free state, involving also loss of nitrogen in the form of ammonia.

3. Very little denitrification, if any, takes place in the roots of diseased spinach. This is evident from the fact that the differences in total, nitrate, amino nitrogen content, etc., of the roots of healthy and diseased plants are usually quite small, running sometimes in the opposite direction.

4. Conditions with regard to peptide and protein nitrogen are apparently somewhat more complicated. In the samples examined the proportion of peptide nitrogen is higher in diseased tops than in normal,

<sup>1</sup> "Ein natürliches System der Gift-Wirkungen," München, 1893, p. 61.

while the proportion of protein nitrogen is higher in diseased roots than in normal, this being also true of diseased leaves when related to the total nitrogen. This is conceivable since the latter is here *smaller* due to loss through denitrification.

5. In round figures, the spinach nitrogen is made up of 55% protein nitrogen, 4.5% diamino nitrogen, 5.5% monoamino nitrogen, and 6% peptide nitrogen. This means that over 70% of the nitrogenous compounds occurring in spinach have direct nutritive value.

WASHINGTON, D. C.

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### NEW BOOKS.

**The Metals of the Rare Earths.** (Monographs on Inorganic and Physical Chemistry.) 1st edition. By JAMES F. SPENCER, B.Sc. (Vict.), D.Sc. (Liverpool), Ph.D. (Breslau), F.I.C., Lecturer in Physical and Inorganic Chemistry at Bedford College (University of London), Reader in Physical Chemistry in the University of London. Longmans, Green & Co.: London, etc., 1919. x + 279 pp., with diagrams. 15 X 22.5 cm. Price, \$4.50 net.

A careful study of this work discloses the fact that the writer is extremely well acquainted with the theory and literature of this group of elements. The book is nicely arranged and is a great advance on many that have appeared in the past. The general plan followed is along similar lines to those of the best kind. The methods of analyses, and the various compounds listed under each element are described at greater length than usual. This in itself is a great recommendation, for it means so much to those who are interested in both research and study upon these metals. It appears unfortunate, however, that there is no chapter devoted to a general discussion of the various types of compounds, for, when we have elements possessing such very similar properties it is very advantageous to consider the salts from a general standpoint by directly viewing tabulated properties, etc.

Another impression gained by reading this book is that it has been written by one who has devoted much more time to the literature than to the practical side, since methods of separation which are absolutely worthless are often placed side by side with the very best without sufficient advice being given to the reader.

In several places we come across statements which indicate without proof that some of the rare earth metals are still complex. This tends to enshroud them in mystery and remove from them their simple nature which has been coming to the front more and more during recent years. It seems better for science that they be considered simple until proved complex beyond a doubt.

This book is so valuable that it is to be hoped that the author will still enlarge the space devoted to the listed compounds and correct all the errors before the next edition appears.

C. JAMES.