cent reaction of this type. The experiments would also tend to lend further color to Dubois'[1] enzyme theory of biophotogenesis. Emmerling[2] has found a number of the ordinary physiologic amino acids among the products of the acid hydrolysis of the *Noctiluca miliaris*, which, as is well-known, is the frequent cause of "phosphorescent" sea-water, but his results do not appear suggestive in this connection. The view has been expressed that in living organisms, the light emission is due to the oxidation of a waste-product and some support to this view might be derived from these experiments.

The writer is indebted to Dr. Jacob Rosenbloom and to Dr. Leonard M. Little, for certain specimens used in these experiments.

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THE OCCURRENCE OF BARIUM IN TOBACCO AND OTHER PLANTS.[3]

BY J. S. MCHARGUE.

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The occurrence of barium in plants was first observed by Scheele[4] in 1788. He obtained barium sulfate from the ashes of beech trees as follows. The ash was leached with distilled water until soluble salts were removed. The insoluble material remaining was digested in a mixture of hydrochloric and nitric acids, filtered and washed. A few drops of sulfuric acid were added and a small white precipitate was thus obtained which Scheele called barium sulfate.

In 1855 Forchammer[5] also detected the presence of barium in the ashes of beech, oak and birch trees. About the same time Eckhard and Boedeker[6] confirmed the presence of barium in beech wood and found it in the sandstone near Göttingen.

In 1874 Knop[7] of Leipzig, while investigating the fertility of the mud carried down by the Nile River, found barium present in samples of the mud collected near the banks and for a considerable distance back on either side of the river. Knop treated 400 grams of the soil with dilute hydrochloric acid, filtered out the insoluble residue and added dilute sulfuric acid to the filtrate. He obtained by this method 0.021 and 0.017% of barium calculated as carbonate.

In the following year, 1875, Dwarzak[8] confirmed the tests made by Knop on the presence of barium in the Nile mud and examined samples

[1] *Orig. Communications, 8th Intern. Congr. Applied Chem.*, **19**, 83–90 (1912).

[2] *Biochem. Z.*, **18**, 372–374 (1909).

[3] Read before the Lexington Section, American Chemical Society, Feb. 12, 1913.

[4] C. G. Scheele, *Opuscula Chemica et physica. Lip.*, 1788, Vol. 1, 258.

[5] *Poggend. Ann.*, **91**, 568 (1854).

[6] *Ann.*, **100**, 294 (1856).

[7] *Landw. Vers. Sta.*, **17**, 65.

[8] *Ibid.*, **17**, 398.

of wheat that had grown in the Nile Valley. In the ash of the leaves and ear he found 0.089% and in the stalk 0.026% of baryta (BaO).

Hornberger[1] in 1899, found in the ash of different parts of the trunks of two copper beech trees, one 102 years and the other 105 years old, 0.97–1.20 in the first and 0.57–0.90% barium sulfate in the second. He also found a small amount of barium in the soil in which the trees grew. He pursued Knop's method in obtaining the barium in the soil, and obtained 9 mg. of barium sulfate from 400 grams of the soil. It is quite probable that he and Knop obtained a very small proportion of the total amount of barium present in the soils on which they worked, since most soils contain sulfate in sufficient amount to precipitate barium when treated with hydrochloric acid.

This includes about all the work that has been done by European investigators on this subject up to the present time.

In this country the work that has been done on the occurrence of barium in plants has been rather recent and confined for the most part to the investigations of the Bureau of Plant Industry and the Bureau of Soils of the Department of Agriculture, in connection with the loco-weed disease of the Southwestern United States.

According to Crawford, the loco-weed, of which there are a large number of species (astragalus mollissimus being one of the commonest), is indigenous to the country from Kansas and Nebraska to California, and from Montana to Mexico and Texas, covering the greater portion of ten states. This plant contains a very peculiar poisonous principle and when eaten a number of times by horses, cattle and sheep, creates a habit for the plant which develops into what is known as the loco disease. In the last stages of this disease various forms of wild excitement are produced, finally terminating in the death of the animal. For more than a half a century various investigators, from time to time, have attempted the isolation of the poisonous principle of the loco-weed and so far without very positive results. Since 1873 the United States Government has been making investigations on this plant and has issued a number of reports concerning it.

In 1908 the Bureau of Plant Industry issued bulletin No. 129 entitled "Barium a Cause of the Loco-weed Disease," by Dr. A. C. Crawford, Pharmacologist for the Bureau. In his investigations upon the loco-weed, barium to the amount of 0.042% as barium sulfate was found in the dry plant. Dr. Crawford concludes that the barium present in the loco-weed is responsible for its poisonous effect on horses, cattle, etc.

In July of 1912 the Bureau of Plant Industry published bulletin No. 246 entitled, "The Relation of Barium to the Loco-Weed Disease," in two parts. In part 1, "A Field Study on the Relation of Barium to the

[1] *Landw. Vers. Sta.*, **51**, 473.

Loco-weed Disease," by C. Dwight Marsh, Plant Physiologist of that Bureau; part 2, "Laboratory Studies on the Relation of Barium to the Loco-weed," by Alsberg and Black, Chemical Biologists for that Bureau.

In part 2 of the report by Alsberg and Black, the results of a number of determinations of barium in plants collected from various parts of Colorado, Wyoming and Arizona are given. They found barium occurring in a large number of plants, in a few of which the barium content is as follows, calculated as $BaSO_4$:

Alfalfa (from Hugo, Colo.)	0.014% in dry plant
Alfalfa (from Limon, Colo.)	0.010% in dry plant
Alfalfa (from Hugo, Colo.)	0.003% in dry plant
Average	0.009% in dry plant
Loco plant from Colo	0.043% in dry plant
Loco plant from Limon, Colo	0.005% min.
Barley, from Colo	0.008% min.
Millet, from Colo	0.006% min.
Beet, from Colo	0.0 % min.

These investigators also collected plants from the proximity of barite veins in Virginia and obtained the following results on same:

	In dry plant.
In broom sedge	0.029% $BaSO_4$
Crab grass	0.029% $BaSO_4$
Soy beans	0.023% $BaSO_4$
Hay	0.014% $BaSO_4$
Broom sedge	0.007% $BaSO_4$
Wheat (seed)	0.0 % $BaSO_4$
Maize (ear, leaf and stem)	0.0 % $BaSO_4$
Maize (ear, leaf and stem)	0.0 % $BaSO_4$

In connection with the study of barium and loco-weed, the Bureau[1] of Soils of the Department of Agriculture have made a large number of barium determinations in the soils from the vicinities in which loco-weed grows. The barium content in 100 samples of soil collected from different parts of Colorado, Wyoming and Kansas, gives an average of 0.06 barium sulfate. The maximum amount present in any one soil was 0.11%. The minimum amount present in any one soil was 0.01%.

The work done by the Bureau of Plant Industry and Bureau of Soils on the occurrence of barium in plants and soils, includes practically all the work that has been done in connection with barium in plants in this country to the present time.

In making the analyses of the leaves of tobaccos for their fertilizing constituents, during the latter part of the summer of 1912, it was observed after ashing 10 grams of the finely ground leaf and treating with an excess of dilute hydrochloric acid, allowing the vessel containing the mixture to remain on the water bath for 30 minutes, filtering and washing

[1] Bureau of Soils, U. S Dept. Agr., *Bull.* 72.

while hot, that after the filtrate had become cool, a slight white turbidity formed at the bottom of each flask. Inasmuch as determinations of calcium, phosphorus and magnesium were all that were desired, and as the solution was distinctly acid, it was decided that no compound of these elements was being precipitated, and the turbidity was passed for the time without further investigation. At the first of January of this year, 1913, another set of tobaccos was prepared for analysis. This lot of tobaccos was of the dark variety and had been grown in Christian County, Kentucky, in 1910, for the purpose of testing the effect of different kinds of fertilizers in the growing of tobacco. The leaves were separated from the stalks and both ground separately for analysis. The usual procedure for ash analysis was carried out until the point after filtering out the insoluble residue in hydrochloric acid and washing was reached. A number of the filtrates were allowed to remain over night undisturbed. On taking up the work next morning a small, white, insoluble precipitate was observed in each flask.

The small white precipitate in one of the flasks was filtered out, washed, ignited and collected on a platinum wire and tested spectroscopically. The spectroscope showed a strong test for barium, with traces of sodium. Inasmuch as barium was lost in the spectroscopic test, another portion of 25 grams of the same sample were ashed and treated with hydrochloric acid as before, filtered, washed and after adding a few drops of dilute sulfuric acid and heating on the water bath for a short time, was set aside and allowed to remain undisturbed for 24 hours. The precipitate thus obtained was only a portion of the total barium present and was designated as precipitate No. 1. That portion of the ash which was insoluble in hydrochloric acid was ignited in a platinum crucible a drop or two concentrated sulfuric acid and about 15 cc. of hydrofluoric acid added and evaporated to dryness over a small flame. A small amount of pure sodium carbonate was then added to the residue and fused. After cooling, the crucible was placed on the water bath and the fused mass extracted with hot water. The insoluble carbonates were filtered and washed with hot water containing 5 grams of c. p. sodium carbonate per liter, until sulfates were removed. The funnel containing the residue of carbonates was suspended in a small Erlenmeyer flask, then covered with a smaller inverted funnel and 5 cc. of warm hydrochloric acid run through the stem of the inverted funnel. After effervescence ceased the funnels and filters were washed, the filtrate placed on the water bath and warm dilute sulfuric acid added, a few drops at a time, until all the barium was precipitated. The barium sulfate thus obtained was combined with the precipitate obtained in hydrochloric acid solution, washed, ignited and weighed for total barium. Similar determinations were made on the leaf and stalk of each sample of tobacco and other plants and gave the following results in per cent. of barium sulfate as indicated in the table:

Tobaccos.

1	Dark variety, Christian Co., Ky.	Leaf	0.071 % $BaSO_4$ in dry plant	
2	Dark variety, Christian Co., Ky.	Stalk	0.041 % $BaSO_4$ in dry plant	
3	Dark variety, Christian Co., Ky.	Leaf	0.030 % $BaSO_4$ in dry plant	
4	Dark variety, Christian Co., Ky.	Stalk	0.039 % $BaSO_4$ in dry plant	
5	Dark variety, Christian Co., Ky.	Leaf	0.065 % $BaSO_4$ in dry plant	1910
6	Dark variety, Christian Co., Ky.	Stalk	0.068 % $BaSO_4$ in dry plant	
7	Dark variety, Christian Co., Ky.	Leaf	0.074 % $BaSO_4$ in dry plant	
8	Dark variety, Christian Co., Ky.	Stalk	0.038 % $BaSO_4$ in dry plant	
9	Dark variety, Ex. Sta. Farm, Ky.	Leaf	0.0096% $BaSO_4$ in dry plant, 1912	
10	Burley Ex. Sta. Farm, Ky.	Leaf	0.014 % $BaSO_4$ in dry plant, 1911	
11	Burley Ex. Sta. Farm, Ky.	Leaf	0.016 % $BaSO_4$ in dry plant, 1911	
12	Burley Fayette Co., Ky.	Stalk	0.009 % $BaSO_4$ in dry plant, 1912	
13	Red Burley Laurel Co., Ky.	Stalk	0.038 % $BaSO_4$ in dry plant, 1912	
14	Standup Burley Ex., Sta. Farm	Stalk	0.016 % $BaSO_4$ in dry plant, 1912	
15	Mammoth Burley, Ex. Sta. Farm	Stalk	0.068 % $BaSO_4$ in dry plant, 1912	
16	Stubs Ex. Sta. Farm	Base	0.150 % $BaSO_4$ in ash, 1912	
17	Stubs Ex. Sta. Farm	Roots	0.115 % $BaSO_4$ in ash, 1912	

Other Plants.

18	Corn stover, Ex. Sta. Farm		0.014 % $BaSO_4$ in dry
19	Corn, Ex. Sta. Farm	Ear and cob	.. % $BaSO_4$ in dry plant, 1912
20	Corn stalks (stubs) Sta, Farm	Base	0.055 % $BaSO_4$ in ash

21	Corn Roots Ex. Sta. Farm	Rootlets	0.022 % $BaSO_4$ in ash
22	Soy beans Ex. Sta. Farm	Whole plant	.. % $BaSO_4$ in dry plant
23	Soy beans, Best White Sta. Farm	Whole plant	0.005 % $BaSO_4$ in dry plant
24	Soy beans, China, Woodford Co.	(Beans)	0.0014% $BaSO_4$ in dry plant
25	Alfalfa, Ex. Sta. Farm	(As cut)	0.0132% $BaSO_4$ in dry plant
26	Hemp Ex. Sta. Farm		0.0036% $BaSO_4$ in dry plant
27	Burdock Root Sta. Farm		0.0440% $BaSO_4$ in ash
28	Poke Root Sta. Farm		.. % $BaSO_4$ in ash
29	Blue Grass Sta. Farm		0.0078% $BaSO_4$ in dry plant
30	Clover Sta. Farm		0.0080% $BaSO_4$ in dry plant
31	Irish potatoes (said to be from Mich.)		0.0160% $BaSO_4$ in ash
32	Wheat (grain) Ex. Sta. Farm		.. % $BaSO_4$ in ash
33	Wheat (grain) West Kentucky		.. % $BaSO_4$ in ash
34	Hickory nut shells, Ky		.. % $BaSO_4$ in dry plant
35	Hazelnut shells, Laurel Co., Ky		0.007 % $BaSO_4$ in dry plant
36	Sycamore stump Ex. Sta. grounds, heart		.. % $BaSO_4$ in ash
37	Sycamore stump last growth, next to bark		0.039 % $BaSO_4$ in ash
38	Banana stalk, after fruit had been removed		0.049 % $BaSO_4$ in dry plant
39	Limestone, Ky. (100 grams)		.. % $BaSO_4$ in rock
40	Coal, Ky		0.014 % $BaSO_4$ in air dry sample
41	Soil, Ky. Ex. Sta. Farm		0.080 % $BaSO_4$ in dry soil
42	Soil, Laurel Co., coal measures		0.042 % $BaSO_4$ in dry soil

The total number of samples thus far examined for barium is 42. Of this number all were plants or parts of plants except 4.

Of the 38 plant samples examined, seven gave negative results. In sample numbered 18, corn stover, grown on the Experiment Station farm, we find 0.014% barium in the stalks and leaves, while in the ears and cobs of the same plant none was found. Sample 22, soy beans grown on the Experiment Station farm, shows no barium, while sample 23, a different variety of bean growing on the same farm, shows 0.005% barium. In sample 24, a bean imported from China and one crop grown in Woodford County, Ky., shows 0.0014% barium in the beans. Sample 28, poke root, grown on the Experiment Station farm, shows no barium. The result on this sample was quite disappointing to the writer, since the poke weed plant is a vigorous one, the roots live for several years and extend from one to two feet below the surface ground. Further experiments will be carried on with this plant to confirm the above result. Samples 32 and 33, wheat (grain) grown in different portions of Kentucky, gave results that confirm Alsberg and Black on wheat grown in Virginia, and in a way contradict the result of Dwarzak on the ash of the ear of wheat grown in the Nile Valley. While Dwarzak seems to have worked on the ear as a whole and not on the corn alone we may infer that the barium obtained by him was in the stalk and chaff of the ear and not in the grain. Sample 34, hickory nut shells, after the kernels had been removed, showed no barium. In samples 36 and 37, interesting results were obtained. Sample 36 represents wood taken from the heart of a large sycamore stump (Platanus occidentalis) about 3 ft. in diameter and about 70 years old, shows no barium. Sample 37 represents wood chipped from the circumference of the same stump and represents the live wood at the time the tree was cut. This sample contained 0.039% $BaSO_4$ in the ash, whereas the heart contained none. We may infer from these two results that the barium is contained in the live portion of the tree and perhaps functions in the vital processes. Further investigations on this point will be carried on for a more positive conclusion.

In the tobaccos two different varieties are represented, the dark and the burley. While there is considerable variation in the amount of barium contained in each variety, it appears from the above results that the dark variety would have a slightly greater average of barium than the burley. However, the two varieties were grown in different seasons and in different soils.

Crawford, Marsh and others in working upon the barium content in the loco-weed, state that in the dry plant the barium is not extracted by distilled water.

In order to test this point in regard to tobacco, a plant containing, in some cases, more than twice the amount of barium reported in any loco-weed by these investigators, 25 grams of the finely ground leaves were digested for 2 hours in 200 cc. of distilled water on the water bath, with an occasional stirring in the meantime. The liquid was filtered and the insoluble material washed until the wash water was of a light straw color. The filtrate was evaporated to dryness in a platinum dish and ashed, taken up in hydrochloric acid, filtered, washed, heated to boiling and a few drops of hot dilute sulfuric acid added and allowed to stand for 24 hours. A precipitate of barium sulfate weighing 0.0025 gram was thus obtained. Two other different samples were similarly treated and gave 0.0017 gram and 0.0024 gram $BaSO_4$. From the results of these experiments we know that a part of the barium contained in tobacco is in a soluble form, and this fact indicates that the soluble barium is in combination with some of the organic acids of the plant and possibly has some function in plant metabolism. This assumption is strengthened by the association of the barium with the live wood cells in the sycamore tree.

The remaining samples not commented on show the occurrence of barium in a somewhat diversified list of plants, other than tobacco, from various sections of this state and country. As an example of the wide occurrence of barium in plants, mention might be made of the potatoes grown in Michigan, a northern state, and the banana stalk from a tropical country. While the majority of the results obtained for this paper are on materials whose barium content has not been previously reported upon, yet there is a sufficient number of determinations on plants whose barium content has been worked upon by various investigators, that show a close agreement in the amount of barium contained in the same plants grown in different parts of the country.

Conclusions.

From the number of barium determinations presented in this paper and those of other investigators, we may derive the following conclusions:

1. That barium in small amounts is widely disseminated through rocks, soils and plants.

2. That in tobacco, a plant whose barium content has not been previously reported upon, the barium varies from the normal content of other plants, both wild and cultivated, to approximately twice the maximum reported in loco-weed.

3. That some of the barium occurring in tobacco can be extracted by distilled water, and is probably in combination with organic acids.

4. The occurrence of barium in the live cells of the higher plants suggests that possibly this metal may function in metabolism.

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SOME DATA ON THE RIPENING OF FLORIDA ORANGES.

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The following data were obtained during the course of a study of some of the commoner enzymes occurring in the peel of the orange, with the view of ascertaining whether any change in the nature or activity of these took place at the point corresponding to the point at which the fruit became sufficiently ripe for human consumption. Previous experiments had seemed to indicate that there might be a point corresponding approximately to the development of the 7 to 1 sugar to acid ratio, where a positive reaction toward some of the ordinary oxidase reagents might be given by the peel though not given previously. This has not been found to be the case.

The fruit was all taken from one tree, at Orlando, Florida, and forwarded by express, the dates of shipment and receipt being given in the table. Tests were made on the day the fruit was received. The writer is indebted to the Orlando Citrus Growers' Association for the fruit used.

The reagents for oxidase activity used were mainly paraphenylenediamine and benzidine; tincture of guaiac, and phenolphthalin were also used, but the former was found rather less reliable than the amino-compounds, and the latter, although reacting readily to enzymes from other sources gave at best only very faint reactions with the orange peel. Aloin was also used. For the peroxidase reagents the same compounds were used, and a few drops of dilute hydrogen peroxide solution were added to the tubes. In most cases Richter's hyperol (urea with hydrogen peroxide of crystallization) was used to prepare the peroxide solution. In no case was oxidase activity detected toward any of the reagents employed, either when applied directly to the peel, or when used with aqueous extracts, either untreated or kept neutral with calcium carbonate during preparation. Strong peroxidase reactions were always obtained, both directly on the peel, and in aqueous extracts. The seeds of the ripe orange give aqueous extracts showing peroxidase reactions to benzidine and to paraphenylene diamine, but not oxidase reactions.

It has also been found that in neutral solution, the water-soluble peroxidase of the peel of the orange will resist heating for 10 minutes to a temperature of 95°, although weakened thereby; the boiling temperature

[1] Florida Citrus Exchange Research Fellow.