

- (b) *The Importance of Gravity Observations at Sea on the Pacific*: JOHN F. HAYFORD, College of Engineering, Northwestern University.
- (c) *A New Method of Measuring the Acceleration of Gravity at Sea*: LYMAN J. BRIGGS, Bureau of Plant Industry, Washington, D. C.
- (d) *The Problem of Continental Fracturing and Diastrophism in Oceanica*: CHARLES SCHUCHERT, Department of Geology, Yale University.
- (e) *The Petrology of Some South Pacific Islands and its Significance*: JOSEPH P. IDDINGS, Brinklow, Maryland.
- (f) *In Relation to the Extent of Knowledge Concerning the Oceanography of the Pacific*: G. W. LITTLEHALES, U. S. Hydrographic Office, Washington, D. C.
- (g) *Marine Meteorology and the General Circulation of the Atmosphere*: CHARLES F. MARVIN, U. S. Weather Bureau, Washington, D. C.
- (h) *On the Distribution of Pacific Invertebrates*: WM. H. DALL, Smithsonian Institution, Washington, D. C.
- (i) *The Marine Algae of the Pacific*: W. G. FARLOW, Department of Botany, Harvard University.
- (j) *The Pacific as a Field for Ethnological and Archaeological Investigation*: J. WALTER FEWKES, Bureau of American Ethnology, Washington, D. C.
- (k) *Mid-Pacific Land Snail Faunas*: H. A. PILSBRY, Academy of Natural Sciences of Philadelphia.
- (l) *Some Problems of the Pacific Floras*: DOUGLAS H. CAMPBELL, Department of Botany, Leland Stanford University.

The symposium contains a summary of some of the results obtained in past exploration of the Pacific and an outline of the importance to many sciences of further systematic and continuous exploration of the Pacific.

15. *Nervous Transmission in Sea-Anemones*: G. H. PARKER, Zoological Laboratory of the Museum of Comparative Zoology at Harvard College.

There is evidence not only for the assump-

tion of independent receptors, but of relatively independent transmission tracts. A first step in the kind of differentiation so characteristic of the nervous organization in the higher animals.

16. *The Responses of the Tentacles of Sea-Anemones*: G. H. PARKER, Zoological Laboratory of the Museum of Comparative Zoology at Harvard College.

The tentacles, in contradistinction to such appendages as those of the arthropods and vertebrates, contain within themselves a complete neuromuscular mechanism by which their responses can be carried out independently of the rest of the animal.

EDWIN BIDWELL WILSON

MASS. INSTITUTE OF TECHNOLOGY

SPECIAL ARTICLES

SOIL BACTERIA AND PHOSPHATES

RAW rock phosphate is by far the cheapest source of phosphorus to apply to soils. It consists chiefly of tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, which is the most common form of phosphorus in the great natural deposits. This phosphorus compound is relatively insoluble in water, and, for this reason, it has been argued by some that it does not become available to plants; but long-continued field experiments, pot-culture experiments, and farm practise have fully demonstrated that this kind of phosphate does become available for plant growth.¹

The increased beneficial results obtained by following the practise commonly recommended of intimately mixing decaying organic matter with the phosphate lead to the suggestion that the action of the soil bacteria that decompose organic matter might be an important factor in the solution of the phosphate.

It has been the common teaching that nitrifying bacteria require the presence of a free base, such as lime or an alkaline carbonate, but we have found that the bacterial action produces acid phosphate and proceeds in the presence of this acid salt.

The importance of the action of decomposition products of the active organic matter of

¹ See Circulars 181 and 186, Illinois Agricultural Experiment Station.

the soil on the solubility of phosphates is better understood by a brief consideration of three important and definitely recognized processes that have long been known to bring about the change of the nitrogen from the unavailable form, as it occurs in the protein of clover, manure, etc., to the readily available form of the nitrate.

(1) *Ammonia Production*.—The first process results in the change of the organic nitrogen to ammonia nitrogen. The ammonia is absorbed by the soil moisture and forms ammonium hydroxid. Much carbon dioxid is produced at the same time and some of it, also, is absorbed by the soil moisture and then unites with the ammonia to form ammonium carbonate, which is alkaline.

(2) *Nitrite Production*.—The second and most important of the three stages consists of the oxidation of the ammonia to nitrite by the nitrite bacteria (*Nitrosomonas*). The oxidation of ammonia to nitrous acid by the nitrite bacteria is represented by the following equation:



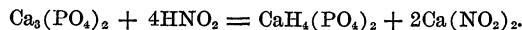
The ammonium portion of the ammonium carbonate has been converted into nitrous acid and carbonic acid has been set free. Both these acids will combine with some base. It is important to note that nitrogen of the alkaline substance, ammonia, has been converted, or transformed, by the biochemical removal of hydrogen and addition of oxygen into a strongly acid substance, nitrous acid.

The primary purpose of this investigation is expressed in the question, Will the calcium of pure rock phosphate, $\text{Ca}_3(\text{PO}_4)_2$, suffice as a base; and, if so, will the phosphorus be made soluble? This will be answered by the experimental data reported in another part of this publication.

If nitrite production takes place with tricalcium phosphate as a source of the base calcium, then the reaction must be represented by one of the following equations:



or



(3) *Nitrate Production*.—The third and last stage is a simple oxidation of the nitrite to nitrate by the action of nitrate bacteria (*Nitrobacter*). It consists in the addition, by biochemical action, of oxygen to the nitrite:



This reaction increases neither acidity nor alkalinity, and no liberation of insoluble compounds would be expected in this process, as no additional base is necessary, as seen by reference to the equation.²

INFLUENCE OF AMMONIA PRODUCTION ON SOLUBILITY OF PHOSPHATES

The most important product formed in the first process, or stage, of the decomposition of organic matter is ammonium carbonate. The ammonium carbonate is alkaline, and consequently could not be expected to exert any action on the solubility of raw rock phosphate.

In 1904 Stalstrom, of Finland, conducted laboratory experiments on the solubility of pure rock phosphates with bacteria which produced ammonium carbonate from peat and from manure containing peat litter. He concluded that there was no appreciable increase in solubility of phosphorus where the bacteria had produced ammonium carbonate over the sterile treatments in which no ammonium carbonate was produced. His experiments lasted forty-two days and were under conditions which would permit of determining soluble phosphorus, were it present. His work is extremely interesting as it demonstrates that in the first stage of decomposition it has been impossible to measure any soluble phosphorus without the growing plant as an indicator.

Similar results have been obtained by the Rhode Island and Wisconsin Experiment Stations in attempts to detect soluble phosphorus in fermenting mixtures of manure and raw

² The results of an experiment to test the effect of the nitrate bacteria on pure tricalcium phosphate support the theory that no solution of phosphorus is to be expected by the action of nitrate bacteria.

rock phosphate and in mixtures of soil and raw rock phosphate.

SOLUTION OF PHOSPHATES BY ACTION OF NITRITE BACTERIA

To determine the part played by the nitrite bacteria in dissolving mineral compounds, and particularly raw rock phosphate, was our principal object in these experiments.

One of the authors made the following suggestion several years ago:³

In the conversion of sufficient organic nitrogen into nitrate nitrogen for a hundred-bushel crop of corn, the nitric acid, if formed, would be alone sufficient to convert seven times as much insoluble tricalcium phosphate into soluble monocalcium phosphate as would be required to supply the phosphorus for the same crop.

The plan of the experiment, briefly stated, was as follows: A thin layer (about $\frac{1}{8}$ inch thick) of a nutrient salt solution was placed in a cone-shaped glass flask of about one liter capacity and about 5 inches in diameter at the bottom. In this solution was placed a definite amount of ammonium salt. The flasks and materials were carefully sterilized. Nitrite bacteria were introduced from pure cultures and sometimes directly from soil. The flasks were plugged with cotton kept at a temperature of 28° C. Many such flasks were prepared, and later, usually at intervals of one week, the contents of two or more flasks were analyzed for nitrogen changed or oxidized and for water-soluble phosphorus and calcium.

In Table I. are shown the relative amounts, by weight, of nitrogen from ammonia sulfate oxidized to nitrite by nitrite bacteria and the amounts of phosphorus and calcium made soluble. Each figure represents the average of duplicate determinations.

EXPLANATION OF RESULTS

The results reported in Table I. demonstrate conclusively that phosphorus and calcium are made soluble while the nitrite bacteria oxidize ammonia nitrogen to nitrite nitrogen. It is also evident that the solubility increases with increasing time of action of the bacteria.

³ Hopkins, "Soil Fertility and Permanent Agriculture," 197.

TABLE I

Phosphorus, Calcium, and Nitrogen Required by Crops, Compared with that Possible of Solution when Nitrite Bacteria Act upon Tricalcium Phosphate
(Expressed in Pounds)

Crop	Nitrogen Required	Phosphorus		Calcium	
		Re- quired	Pos- sible	Re- quired	Pos- sible
Corn:					
Grain, 100 bu.; Stover, 3 tons; cobs, $\frac{1}{2}$ ton.....	150	23	166	22	321
Wheat:					
Grain, 50 bu.; straw, $2\frac{1}{2}$ tons..	96	16	107	11	206
Oats:					
Grain, 100 bu.; straw, $2\frac{1}{2}$ tons.	97	16	108	17	208
Timothy, 3 tons.....	76	9	84	20	163

An inspection of the figures shows that there is, by weight, approximately twice as much phosphorus and four times as much calcium made soluble as there is nitrogen oxidized by the bacteria. As an average of the results from thirteen flasks (Nos. 4 to 16), we find that from the oxidation of 56 pounds of nitrogen 115 pounds of phosphorus and 211 pounds of calcium are made soluble. (The results from Flasks 1, 2 and 3 are not included in the ratio calculated.) According to theory, when 56 pounds of nitrogen are changed from the ammonia form to the nitrite form, with both the nitrous acid (HNO_2) and the associated sulfuric acid (H_2SO_4) acting on the pure rock phosphate, 124 pounds of phosphorus and 240 pounds of calcium are made soluble.

Ten cubic centimeters of Flask 16 required 3.35 cc. of N/12.5 NaOH with phenolphthalein as the indicator for the second hydrogen atom. The normality of the solution was found to be N/37.2.

IMPORTANCE AND EXTENT OF THE ACTION OF NITRITE BACTERIA

It has already been shown that the nitrite bacteria make phosphorus and calcium soluble from pure rock phosphate and that the action conforms to a definite chemical ratio.⁴

⁴ It was found that the action of the nitrite bacteria was the same on the natural raw rock

The nitrous acid produced may act upon compounds of iron, aluminum, potassium, sodium, or magnesium which occur in soils, or it may act upon tricalcium phosphate, calcium silicate, or calcium carbonate, if present. For this reason, it has been recommended that the ideal practise to obtain the greatest solubility of the raw rock phosphate is to turn it under in intimate contact with organic matter, and, if needed, to apply ground limestone after plowing or at some other point in the crop rotation.

In Table II. are presented the actual amounts of phosphorus, calcium and nitrogen required by standard crops, and the amounts of phosphorus and calcium which would be made soluble if all the nitrogen required for the crop should be oxidized to nitrate and should act upon pure rock phosphate.

TABLE II
Nitrogen Oxidized, and Phosphorus and Calcium Made Soluble by Nitrite Bacteria
(Expressed in Milligrams)

Flask	Duration in Days	Nitrogen Oxidized	Phosphorus Made Soluble	Calcium Made Soluble
1	28	2.54	4.08	3.87
2	41	4.81	5.08	5.60
3	41	...	5.99	8.40
4	48	5.52	9.56	14.80
5	48	4.88	10.20	18.40
6	55	6.40	12.85	22.00
7	55	6.40	10.24	23.52
8	62	6.88	16.00	31.04
9	48	3.61	7.52	13.60
10	62	3.87	8.76	16.48
11	62	5.84	9.82	16.00
12	62	5.68	11.28	20.80
13	69	6.03	11.14	22.40
14	48	5.76	13.04	24.80
15	69	4.60	11.60	19.20
16	139	18.84	41.56	75.26

The figures show that there is possible of solution from this biochemical process about 7 times as much phosphorus as corn, wheat or oats require, and 9 times as much as timothy requires. Greater differences occur in the calcium figures, there being possible of solution phosphate as on the pure rock phosphate, but more extensive experiments with the natural rocks will be reported later.

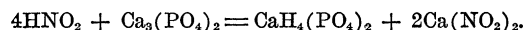
14 times that required for corn, 18 times that required for wheat, 12 times that required for oats, and 8 times that required for timothy.

SUMMARY

1. Nitrite bacteria make phosphorus and calcium soluble from insoluble phosphates when they oxidize or convert ammonia into nitrite.

2. The actual ratio found shows that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized, aside from the action of the acid radicles associated with the ammonia.

3. The ratio of solubility found on the basis of nitrogen to phosphorus and calcium conforms to the following reaction:



According to this equation, 56 pounds of nitrogen liberate in soluble form 62 pounds of phosphorus and 120 pounds of calcium.

4. Neither ammonia-producing bacteria nor nitrate bacteria liberate appreciable amounts of soluble phosphorus from insoluble phosphates.

More complete details of these experiments will be published in Bulletin No. 190 of the University of Illinois Agricultural Experiment Station.

CYRIL G. HOPKINS,
ALBERT L. WHITING

UNIVERSITY OF ILLINOIS

THE AMERICAN CHEMICAL SOCIETY II

The following papers were read and discussed.

The So-called Caseinates: W. D. BANCROFT.

Action of Rennin on Caseine: W. D. BANCROFT.

The Aeration Method for Total Nitrogen Determinations: R. S. POTTER AND R. S. SNYDER.

Titrimetric Determination of Nitrite N: B. S. DAVISSON.

Determination of Ammonia by Aeration: B. S. DAVISSON.

A Study of Carbohydrates as Milk Modifiers: RUTH WHEELER.

The Relation of a Diet High in Calcium to the Calcium Content of Tissues: AMY L. DANIELS.