$P_f$ was increased and finally a constant transport for large $P_f$. Their results are shown in Fig. 2. Their experimentally determined oxygen-hemoglobin dissociation curve is given as the dashed curve in Fig. 1. We readily see that these results are in qualitative agreement with Eq. 17. When $P_f$ is zero $f(kP_i) - f(kP_f)$ takes its maximum value, and as $P_f$ is increased to the point where the hemoglobin is largely saturated, $f(kP_i) - f(kP_f)$ decreases, monotonically approaching zero. As the second term in Eq. 17 becomes negligible, all the oxygen transport is due to dissolved gas and is therefore independent of $P_f$.

We feel that these considerations make it clear, by and large, how hemoglobin is effective in oxygen transport. That is, if the hemoglobin is under conditions such that it is saturated with oxygen at both ends of the transport path, it cannot be effective at all.

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**References**

1. P. F. Scholander, *Science* **131**, 585 (1960).
2. E. Hemmingsen and P. F. Scholander, *ibid.* **132**, 1379 (1960).
3. D. R. Olander, *A.I.Ch.E. Journal* **6**, 233 (1960).
4. J. H. Comroe, R. E. Forster, A. B. DuBois, W. A. Briscoe, E. Carlsen, *The Lung* (Year Book Publishers, Chicago, 1955), p. 98.

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## Effects of Plant Nutrients on Uptake of Radiostrontium by Thatcher Wheat

*Abstract.* The effects of various dosages of ammonium dihydrogen phosphate, monocalcium phosphate, calcium chloride, and potassium chloride on the uptake of radiostrontium by Thatcher wheat grown in Saskatchewan Oxbow loam soil containing strontium-85 were studied. Monocalcium phosphate at a dose level of about 600 lb/acre of soil effected a statistically significant reduction of strontium-85 uptake in each of the four plant fractions of grain, chaff, stem, and leaf. At the very reasonable dosage of about 60 lb/acre, monocalcium phosphate gave a statistically significant reduction in strontium-85 uptake in the grain and chaff.

In 1958, Libby (*1*) reported that addition of solutions of potassium salts to a Washington garden soil containing $Sr^{90}$ had a beneficial effect in reducing the uptake of the $Sr^{90}$ by radish plants. His conclusions were criticized (*2*) because the soil was not fully characterized and the experiments lacked sufficient replication. There is no dispute, however, on the need for more research on methods of soil treatment which may lead to a reduction in plant uptake of $Sr^{90}$ (*3*). Recently, Fowler and Christenson (*4*) described a study on the effect of soil nutrients on plant uptake of fallout, and reported that increasing the concentration of soil calcium has a depressing effect on the concentration of $Sr^{90}$ per milligram of calcium in lettuce, grass, and alfalfa. In an investigation with bean plants on the relative availability of a number of $Sr^{90}$ compounds in soil, Uhler and Hungate (*5*) noted the low availability of strontium phosphate, especially in calcareous soils, thus suggesting the possibility of the use of phosphate fertilizers as a means of reducing $Sr^{90}$ uptake. However, it was stated by these authors (*5*) that, although their results indicated that uptake of $Sr^{90}$ might potentially be reduced through precipitation reactions, initial experiments with phosphate fertilizers did not permit much optimism on this point.

As an extension of our earlier work on the uptake and distribution of $Sr^{90}$ in Thatcher wheat (*6*), the effects of a number of plant nutrients on the uptake of $Sr^{85}$ by Thatcher wheat were investigated (*7*). The plants were grown, three per pot, in the greenhouse. Each pot contained 400 g of Saskatchewan Oxbow loam soil which had a *p*H of 7.2, and 19.2, 6.5, 1.9, and 0.1 meq of exchangeable Ca, Mg, K, and Na, respectively, per 100 g of soil. Before the seeds were sown, each pot of soil was first mixed thoroughly with 50 ml of $Sr^{85}$ (*8*) solution (1 $\mu$c/ml) and then with 50 ml of nutrient solution or with 50 ml of distilled water as a control. The plant nutrients, used at various dose levels as given in Table 1, were ammonium dihydrogen phosphate, monocalcium phosphate, calcium chloride, and potassium chloride. Each treatment was repeated in six pots. The seeds were germinated, and the plants were grown to maturity. The soil moisture was kept at field capacity by daily watering. After harvest, individual plants were separated into grain, chaff, stem, and leaf and analyzed for radioactivity. Since three plants were grown per pot and each treatment was repeated in six pots, analyses from 18 replications were obtained.

Table 1. Uptake of strontium-85 by Thatcher wheat grown in Saskatchewan Oxbow loam soil. Each value for uptake is the mean of 18 replications.

| Nutrient treatment | Dosage (meq/100 g of soil) | Uptake (%) per gram of dried tissue | | | |
|---|---|---|---|---|---|
| | | Grain | Chaff | Stem | Leaf |
| None (control) | | 0.0106 | 0.026 | 0.071 | 0.376 |
| $NH_4H_2PO_4$ | 0.08 $PO_4$* | .0116 | .027 | .071 | .378 |
| | 0.16 $PO_4$ | .0100 | .026 | .065 | .323† |
| | 0.80 $PO_4$ | .0101 | .031† | .066 | .338 |
| $Ca(H_2PO_4)_2$ | 0.08 $PO_4$, 0.027 Ca* | .0088† | .021† | .070 | .399 |
| | 0.016 $PO_4$, 0.054 Ca | .0089† | .026 | .073 | .390 |
| | 0.80 $PO_4$, 0.27 Ca | .0078† | .023† | .061‡ | .324† |
| $CaCl_2$ | 0.027 Ca | .0090† | .025 | .068 | .349 |
| | 0.27 Ca | .0097 | .025 | .062 | .343 |
| KCl | 1.9 K§ | .0092 | .021† | .059 | .351 |
| | 3.8 K | .0103 | .023† | .065 | .386 |
| L.S.D.‖ | | .0016 | .002 | .009 | .047 |

* This dosage is equal to a treatment of about 60 lb. of nutrient per acre of soil, assuming the weight of 1 acre of soil to be $2 \times 10^6$ lb. † Significant at the 1 percent level. ‡ Significant at the 5 percent level. § This dosage is equal to the amount of exchangeable potassium already present in the soil. ‖ Least significant difference.

The separated plant tissues were ashed in a muffle furnace. Each sample of ash was dissolved in 1 ml of hot aqua regia, and when necessary, as in the case of the ash from leaves, a few drops of hydrofluoric acid were added to bring any siliceous residue into solution. Each dissolved sample was rinsed into a plastic tube and made up to a standard volume of 5.0 ml with an aqueous solution containing 1 mg of $SrCl_2$ per milliliter. The $Sr^{85}$ activity was then determined in a well-type liquid scintillation counter. The data were analyzed statistically (*9*). The results are summarized in Table 1.

Inspection of Table 1 shows that ammonium dihydrogen phosphate, calcium chloride, and potassium chloride, when applied at dose levels used in these experiments, did not give any consistent pattern in reducing $Sr^{85}$ uptake. It may be pointed out that as a means of expressing the data in a uniform way, the accumulations of strontium are given in Table 1 as the percentage uptake per gram of dried tissues instead of total uptake in various parts of the plant. With only one exception, the various nutrient treatments did not influence average yields by more than a few percent as judged by the weights of the grain. The exception was the treatment with the highest dose of ammonium dihydrogen phosphate, where an increase in average yield of about 30 percent was noted. This may bring in a carbohydrate dilution factor when the uptake of strontium per gram of dried tissue was calculated. However, statistical analysis of the data expressed as the percentage of total uptake also showed that treatments with ammonium dihydrogen phosphate did not cause any statistically significant difference from total uptakes in the control. Of the ten nutrient treatments given in Table 1,

only monocalcium phosphate, a component of "superphosphate" fertilizer, applied at the highest dose level of about 600 lb/acre, effected a statistically significant reduction of $Sr^{85}$ uptake in all four fractions of the wheat plant. Even at the very reasonable dosage of about 60 lb/acre, monocalcium phosphate gave a statistically significant reduction in $Sr^{85}$ content in the grain and chaff. These results appear to indicate that a combined utilization of both the calcium effect (*4*) and the phosphate effect (*5*) may have some promise as a means of soil treatment to reduce plant uptake of radiostrontium.

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**References and Notes**

1. W. F. Libby, *Science* **128**, 1134 (1958).
2. L. A. Romo, *ibid.* **129**, 864 (1959).
3. W. F. Libby, *ibid.* **129**, 866 (195 → J. L. Kulp, R. Slakter, A. R. Schubert, *J. Agr. Food Chem.* **7**, 466 (1959).
4. E. B. Fowler and C. W. Christenson, *Science* **130**, 1689 (1959).
5. R. L. Uhler and E. P. Hungate, *Nature* **187**, 252 (1960).
6. C. C. Lee, *Science* **129**, 1280 (1959).
7. This work was generously supported by the Saskatchewan Research Council and the Saskatchewan Agricultural Research Foundation.
8. Strontium-85 solution was supplied by Oak Ridge National Laboratory. The specific activity was designated as greater than 500 mc/g.
9. Sincere thanks are extended to Dr. F. Sosulski for doing the statistical analysis.

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## Vitamin $B_{12}$ Requirement of a Marine Blue-Green Alga

*Abstract.* A species of *Synechocystis* isolated from a marine mud has an absolute requirement for vitamin $B_{12}$. Analogues of $B_{12}$, including cobinamide and α-(adenyl) cobamide cyanide, satisfactorily substitute for vitamin $B_{12}$. Methionine alone or in combination with other compounds supports only a low level of growth.

Recent discoveries that many phytoplankton organisms require vitamins (*1*), notably $B_{12}$, point to vitamin cycles as significant determinants of the productivity of the sea. Blue-green algae are of widespread occurrence in marine environments and are undoubtedly important contributors to the productivity of these areas. Despite this, little is known of their growth and nutrition. A blue-green alga belonging to the genus *Synechocystis* (termed 17a in our laboratory), isolated from a mud sample from Long Island Sound, has been found to have an absolute requirement for vitamin $B_{12}$. Its $B_{12}$ requirement and possible sparing action of certain compounds were investigated in detail.

The ASP-2 medium of Provasoli (*2*), modified by increasing phosphate 10-fold and nitrate 20-fold, initial *p*H 8.2, has been found suitable for the enrichment and isolation of many marine blue-green algae, and for subsequent growth studies. The *Synechocystis* was isolated in pure culture by repeated pour plates of the modified ASP-2 medium plus 2 percent agar.

Growth studies were carried out with a less elegant modification of the test-tube culture method of Myers (*3*). The organism was grown in test-tubes in 10 ml of modified ASP-2 medium in a thermostatted bath at 37°C with 1 percent $CO_2$ in air bubbling through the tubes. Growth was measured as optical density at 600 mμ, with a Bausch and Lomb Spectronic 20. The optical density was reasonably linear with cell concentration up to an optical density of 1.0. At this density the cell volume was 0.68 $mm^3$/ml.

Growth is expressed in terms of the specific growth rate $k$ with the dimensions of $\log_{10}$ units per day (*4*), or as the optical density reached in an arbitrary time long enough to show effects of materials which become limiting, but which are present in sufficient amount to allow equivalent growth rates up to an optical density of 1.0. Optical density readings $> 1.0$ were obtained by diluting the suspension with fresh medium. The $B_{12}$ analogues were diluted from stock solutions (*5*). Table 1 shows the growth response of *Synechocystis* to $B_{12}$, $B_{12}$ analogues, and methionine.

Experiment 1 shows that the $B_{12}$ requirement is absolute and demonstrates the response to increasing $B_{12}$. Methionine alone supported only limited growth. Experiment 2 shows the response to various $B_{12}$ analogues. Growth was equally good on all the analogues tested except the two analogues with adenine substituted in the 2-position. With these two compounds the growth rate and the total growth in 40 hours were very low, possibly reflecting a steric effect which causes adenyl $B_{12}$ compounds substituted in the 2-position to be converted only with difficulty to the coenzyme form within the cell.

Several compounds and combinations were tried for sparing action of the $B_{12}$ requirement for *Synechocystis*. Deoxyribosides, S-3 vitamin mix (*2*), choline, sarcosine, RNA, DNA, and DNA hydrolyzed with formic acid allowed no growth. Casamino acids and yeast extract allowed only slight growth at 40 hours. Soytone was somewhat better.

With $B_{12}$ at a saturating level for growth, the addition of Casamino acids or yeast extract caused no increase in growth rate. The addition of Soytone with $B_{12}$ resulted in some inhibition of growth.

Table 1. Response of a species of *Synechocystis* (organism 17a) to $B_{12}$, methionine, and $B_{12}$ analogues. $B_{12}$ nomenclature is taken from *J. Am. Chem. Soc.* **82**, 5575 (1960). OD, optical density.

| Addition | $k$ | OD at 40 hr |
|---|---|---|
| *Experiment 1* | | |
| No $B_{12}$ | 0 | 0.027 |
| $B_{12}$, 0.05 μg/lit. | 1.82 | 1.52 |
| $B_{12}$, 0.1 μg/lit. | 2.18 | 3.62 |
| $B_{12}$, 0.5 μg/lit. | 2.10 | 7.45 |
| $B_{12}$, 1.0 μg/lit. | 2.20 | 7.70 |
| DL-Methionine, 20 μg/ml | | 0.147 |
| DL-Methionine, 200 μg/ml | | .268 |
| *Experiment 2* | | |
| No $B_{12}$ | 0 | 0.007 |
| α-(5, 6-Dimethylbenzimidazolyl) cobamide cyanide, 1.0 μg/lit. | 2.30 | 5.09 |
| α-(2-Methyladenyl) cobamide cyanide, 2.0 μg/lit. | | 0.96 |
| α-(Adenyl) cobamide cyanide, 2.0 μg/lit. | 2.36 | 7.21 |
| Cobinamide, 2.0 μg/lit. | 2.20 | 5.09 |
| α-(5-Hydroxybenzimidazolyl) cobamide cyanide, 2.0 μg/lit. | 2.36 | 6.20 |
| α-(2-Methylmercaptoadenyl) cobamide cyanide, 2.0 μg/lit. | | 0.27 |
| α-(5-Methyladenyl) cobamide cyanide, 2.0 μg/lit. | 2.20 | 5.53 |
| α-(Benzimidazolyl) cobamide cyanide, 2.0 μg/lit. | 2.36 | 4.95 |

Various 6-substituted pteridines known to occur in blue-green algae (*6*), including pteridines isolated from *Synechocystis*, were tested singly and together with methionine for possible sparing action. None had any marked effect except 2,6-diamino-4-hydroxypteridine (*7*), which, in the presence of methionine, allowed twice as much growth in 40 hr as methionine alone.

In common with many other marine algae and protozoa (*8*), it appears that marine blue-green algae will also show a requirement for exogenous $B_{12}$. The red pigmented marine blue-green alga, *Phormidium persicinum*, requires $B_{12}$ (*9*). An examination of the vitamin requirements of other marine blue-green algae recently isolated in this laboratory has shown that more than 50 percent also require $B_{12}$. Whether this requirement for a vitamin and possibly utilization of other organic materials reflects a greater degree of heterotrophy in marine blue-green algae than in the fresh water blue-green algae presently known remains to be seen (*10*).

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**References and Notes**

1. L. Provasoli, *Ann. Rev. Microbiol.* **12**, 279 (1958).
2. L. Provasoli, J. J. A. McLaughlin, M. R. Droop, *Arch. Mikrobiol.* **25**, 392 (1957).
3. J. Myers, in *The Culturing of Algae* (Kettering Foundation, Yellow Springs, Ohio, 1950), pp. 45–51.