sures of culture solutions obtained by the addition of sucrose bring about a marked reduction in the entrance of water into a plant has been demonstrated by Tagawa (11), Rosene (9), and Hayward and Spurr (4). According to Long (6) the addition of sucrose to the culture solution greatly reduced water entrance into the roots of tomato plants but did not greatly disturb mineral entrance. Spoehr (10) and Went and Carter (12) found that sucrose was not absorbed through roots and its effect on growth was negligible. In direct contrast Dormer and Street (1) studying carbohydrate nutrition of tomato roots postulated a phosphorylation of sucrose with consequent entrance into plant cells. Hanson and Biddulph (2) studied the effect of added sucrose on the translocation of absorbed ions. They found that in darkness the addition of sugar to the culture solution had no significant effect on the translocation of phosphate to the shoots, but that in the daytime the translocation of phosphate did increase. This latter phenomenon was attributed to the increase in photosynthetic sugar and not to the sugar in the culture solution. The autoradiograms obtained from the plants whose roots were in contact with sucrose in the experiment here recorded show no enhanced absorption of phosphorus. Quite to the contrary, there is a reduced amount of P^{32} in the leaves of these plants, a condition attributed to a lack of transport of P^{32} to the tops of the plants because of a reduction in water flow through the xylem.

After two months of growth in a complete culture solution sunflower plants were transferred to culture solutions containing 275 μc P^{32}/500 ml and allowed to remain in contact with the P^{32} for 90 minutes. Transpiration was varied by light vs darkness, different relative humidities, and different osmotic pressures of the culture solution obtained by the addition of sucrose. The distribution of P^{32} in the leaves was recorded by means of autoradiograms. It was noted that whatever means was used to vary the amount of transpiration, and, therefore, the amount of water absorbed and transported upward through the plants, there was a positive relationship between water transport and the amount of P^{32} accumulation in the leaves, higher rates of water loss being associated with greater amounts of P^{32} in the leaves.

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STUDIES ON NITRITE METABOLISM IN HIGHER PLANTS[1]

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The theory that nitrate reduction proceeds in a stepwise manner as follows

$$NO_3^- \rightarrow NO_2^- \rightarrow HNO \rightarrow NH_2OH \rightarrow NH_3$$

has received much support recently with the isolation of nitrite reductase by Evans and Nason (2), the isolation of hydroxylamine reductase by Zucker and Nason (7) and the demonstration of a nitrite reductase system which reduces nitrite to ammonia (4). In this report manometric experiments with nitrite and experiments with $N^{15}O_2^-$ are presented to show further evidence that nitrite is an intermediate in nitrate reduction.

During manometric studies to determine the effect of nitrite on the processes of respiration and photosynthesis it was observed that leaves infiltrated with nitrite and exposed to light rapidly evolved a gas and caused a simultaneous disappearance of nitrite. Investigation showed that the gas was almost entirely oxygen and that the amount of oxygen evolved was

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[2] Charles F. Kettering Foundation Predoctoral Fellow.

TABLE I

FATE OF NITRITE SUPPLIED TO WHEAT LEAVES

	MICROMOLES	%
$N^{15}O_2^-$ taken up	37.0	100.0
$N^{15}O_2^-$ unchanged	1.0	2.7
$N^{15}O_2^-$ reduced to amino level	30.5	82.5

directly proportional to the amount of nitrite disappearing.

To determine the fate of the nitrite supplied to wheat leaves the following experiments were performed. One-gram samples of wheat leaves from 10- to 12-day-old wheat seedlings were placed with the cut ends in 10 ml of 0.01 M $KN^{15}O_2$. The $KN^{15}O_2$ was synthesized from $KN^{15}O_3$ according to a standard procedure for reducing nitrate to nitrite (3). The wheat leaves were exposed to light (tungsten filament) of 5000 fc intensity for four hours at 30° C, with air flowing rapidly over them. The leaves were extracted with hot 80 % ethanol, and nitrite and nitrate determined together by the method of Varner et al (6). Nitrite was determined in the original solution to establish the amount of $N^{15}O_2^-$ entering the leaves. Reduced nitrogen fractions (ammonia, amide, amino acid and proteins) were recovered in one sample by the Kjeldahl procedure. The ammonium sulfate from each of these three samples was converted to gaseous nitrogen by the method of Rittenberg (5) for determination of N^{15} by the mass spectrometer. Table I shows the results from these experiments.

These results clearly show that wheat leaves can reduce nitrite to the amino level of reduction. Since we have never observed a significant increase in the levels of ammonia and amides in the wheat leaves during the reduction of nitrite, the major part of the reduced nitrogen is probably being used in protein synthesis. It is of course understood that although a plant may be able to reduce nitrite, the normal reduction of nitrate by that plant does not necessarily proceed through the nitrite stage. However, the isolation of an enzyme capable of reducing nitrate to nitrite (2) lends support to this hypothesis.

It is interesting to note in table I that very little nitrite accumulates in the wheat leaves, that is, it is reduced as rapidly as it enters the leaf. This is in contrast to the behavior of nitrate which will enter excised wheat leaves much more rapidly than it is reduced (1). It is difficult to say whether this difference in rates of reduction is due to a difference in rates at which the two ions can arrive at the reduction site or is due to a difference in capacities of the enzymatic systems involved.

TABLE II

CORRELATION BETWEEN NITRITE DISAPPEARANCE AND GAS EVOLUTION

NITRITE DISAPPEARED	GAS EVOLVED	MOLE RATIO
micromoles	*micromoles*	
2.3	3.1	1.34
2.4	3.5	1.46

TABLE III

EFFECT OF ETIOLATION ON LIGHT * REDUCTION OF NITRITE

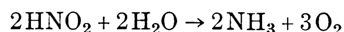
LEAVES	CHLOROPHYLL CONTENT	NITRITE REDUCED
	mg/gm	*micromoles/gm*
Green	2.47	0.72 **
Etiolated	0.0	0.04 **
Etiolated + 2 hrs light	0.18	0.70 **

* Intensity, 500 fc.

** Each value represents the average of 2 or 3 determinations.

The quantitative relationship between the nitrite disappearing and the gas evolved during the illumination of wheat leaves was established in the following manner. A one-gram sample of wheat leaf sections (1 cm long) was vacuum infiltrated with 0.05 M KNO_2 and divided into two equal parts. One part was placed in a Warburg flask and illuminated for 20 minutes at 30° C, at 2000 fc. The other part of the sample was used as a dark control. The rate of reduction in the dark was usually less than 5 % of the rate in the light. The difference between the quantities of nitrite reduced in the light and the dark was used in calculating the ratio of nitrite disappearing as gas evolved. Typical data are shown in table II. Standard gas analysis tests showed that the gas evolved was oxygen with a little nitrogen in it.

If nitrite were reduced by the overall reaction



$$2HNO_2 + 2H_2O \rightarrow 2NH_3 + 3O_2$$

the ratio of oxygen produced to nitrite reduced would be 1.5. From the data in table II it can be concluded that the photolysis of water is the primary and immediate source of reducing substance used in the light reduction of nitrite by wheat leaves.

In experiments similar to these, wheat leaves were infiltrated with $KN^{15}O_2$ and the gas evolved analyzed for N_2^{15}. This gas always contained some N_2^{15} and in one experiment contained as much as 18 % N_2^{15}. Presumably this results from the reaction of nitrite with the amino nitrogen of the leaves.

The dependence of the light reduction of nitrite (measured by nitrite disappearance) on chlorophyll is shown in table III.

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FLOWER-PROMOTING ACTIVITY OF PEA SEED DIFFUSATES [1,2]

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It has been established that certain varieties of peas can be vernalized (3). Cold treatment of the germinating seeds of such pea varieties induces a number of changes in the subsequent growth of the seedlings at normal temperature. Of these changes one of the most interesting is an acceleration of flowering. The physiological mechanisms by which such cold treatment affects the seed have, however, remained obscure. In this paper it will be shown that an extract may be prepared from pea seeds which may be used to replace low temperatures in the treatment of other seeds. This extract, like low temperatures, is effective in reducing the number of nodes to the first flower in non-cold treated but vernalizable pea varieties.

Pea diffusates of seeds were prepared by a modification of the method of Bonner, Haagen-Smit and Went (1): one end of a glass cylinder 5 cm long and 7.5 cm in diameter was wrapped in gauze and put into a 600-ml beaker. The beaker and the cylinder, immersed in glass distilled water, were covered with paper toweling and sterilized. Dry seeds were sterilized by immersion for 10 min in a 0.05 % solution of sodium hypochlorite.

They were then washed four to six times with sterile distilled water. About 80 sterile seeds were next put on the gauze and sufficient water put in the beaker so that when the seeds had become completely turgid they were partially immersed in the remaining liquid. The beakers were covered with paper toweling. The beakers containing the seeds were then subjected to the following conditions: 1) 4° C in dark for 25 days; and 2) 23° C in dark for 5 days. At the end of these treatment periods, the germinating seeds were removed and planted. Most of these plants did not survive; those that did, were damaged and could not be examined for flowering. The liquid (diffusate) which remained in the beakers was used for soaking fresh dry seed. The period of soaking in the diffusate was approximately 10 hours at 20 to 23° C. The plants after soaking were grown under conditions of a 24-hour cycle made up of eight hours natural light at 20° C, eight of artificial light at 17° C, and eight hours dark at 17° C.

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TABLE I

EFFECT OF PEA DIFFUSATES ON THE SUBSEQUENT FLOWERING OF PEAS

TREATMENT	MEAN NODE TO FIRST FLOWER
Control (H_2O)	20.2 ± 0.54
4° C Diffusate	18.4 ± 0.828 *
23° C Diffusate	17.85 ± 1.34 *

Diffusates made at low (4° C) or high (23° C) temperatures. Seeds soaked for 10 hrs in these diffusates or in distilled H_2O as the control and then grown at high temperature (20° C day, 17° C night).
* Differences from control significant at the 1 % level.

Table I shows the data of one of a series of experiments in which dry seeds were soaked either in the diffusate or in water. It can be seen that both diffusates, i.e., from 4° C (vernalized) and 23° C (unvernalized) seed, are effective in reducing the number of nodes to the first flower.

The data show that the diffusate contains an active principle capable of replacing cold treatment for this variety of pea which is normally quantitatively vernalizable. Diffusates were found to be inactive in the Avena section growth test, and it is therefore unlikely that the active principle is an auxin. It may however be a flowering hormone or possibly a precursor of a flowering hormone.

Earlier work, summarized by Fries (2) has shown that pea diffusates contain a substance or substances which causes appreciable growth of excised leaves. Removal of the cotyledons from otherwise normal etiolated pea seedlings resulted in a marked decrease in the subsequent growth of the leaves.

The present studies indicate the presence of still another factor in pea diffusates, namely a factor active in the promotion of flowering.

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