2,5-Dimethoxy-2,5-dihydrofurfural (III). I (3.01 g, 0.116 mole) was dissolved in methanol (10 ml) and the solution added to a solution of sodium methoxide (from 0.266 g of sodium; 0.0116 mole) in methanol (15 ml). After standing at room temperature for 15 minutes the methanol and the methyl acetate formed by the reaction were distilled in a vacuum. Ether (50 ml) was added to the colorless residue, a precipitate of sodium acetate (0.88 g = 93 %) removed by filtration and the ether solution distilled. The yield was 1.40 g (77 %) of III (colorless liquid, b.p.$_{10}$ 88°, n_D^{25} 1.4430) (Found: C 53.3; H 6.8; OCH_3 39.6. Calc. for $C_5H_6O_2(OCH_3)_2$ (158.2): C 53.2; H 6.4; OCH_3 39.3).

On standing the product changes into a more viscous liquid with a higher refractive index. Probably polymerization takes place. Redistillation of 1.20 g after 10 days gave 0.74 g of the original product (b.p.$_{20}$ 98°, n_D^{25} 1.4431).

2,5-Dimethoxytetrahydrofurfural diacetate (IV). I (2.34 g) was dissolved in dioxan (50 ml) and the solution shaken (3 hr) with Adams platinum oxide catalyst (100 mg) under hydrogen (70 atm). The product was isolated by distillation. The yield was 1.92 g (81 %) of IV (colorless liquid, b.p.$_{0.3}$ 97—99°, n_D^{25} 1.4398) (Found: C 50.7; H 7.2; OCH_3 23.5; $COCH_3$ 31.8. Calc. for $C_5H_6O_3(OCH_3)_2(COCH_3)_2$ (262.3): C 50.4; H 6.9; OCH_3 23.7; $COCH_3$ 32.8).

2,5-Dimethoxytetrahydrofurfural (V). IV (3.00 g) was treated with sodium methoxide as described above for the preparation of III. The yield was 1.40 g (77 %) of V (slightly yellow liquid, b.p.$_{14}$ 82—84°, n_D^{25} 1.4307) (Found: C 52.9; H 7.9; OCH_3 38.9. Calc. for $C_5H_6O_3(OCH_3)_2$ (160.2): C 52.5; H 7.6; OCH_3 38.7).

The following day the refractive index of the product had risen to 1.4323. Redistillation of 1.23 g gave 1.02 g of the original product (colorless liquid, b.p.$_{14}$ 82—83°, n_D^{25} 1.4306). Apparently V, like the corresponding dihydrofuran III, polymerizes on standing.

Reaction of liquid dimethoxydihydrofurfural diacetate with sodium methoxide. A portion (15.0 g) of the mother liquor from the isolation of I was treated with sodium methoxide in methanol and the red reaction mixture worked up as described for the preparation of III. The yield was 0.82 g (9 %) of III (b.p.$_{11}$ 93—94°, n_D^{25} 1.4450) (Found: C 53.0; H 6.6; OCH_3 39.2).

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Fixation of Nitrogen by Nodules Excised from Illuminated and Darkened Pea Plants

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In the symbiotic nitrogen fixing system of leguminous plant and root nodule bacteria, the photosynthesizing plant must translocate substrates to the nodule to support its general respiration and growth, to supply energy for nitrogen fixation, and to accept the fixed nitrogen. There is little information to indicate how rapidly these substrates are exhausted after photosynthesis ceases. Lindstrom, Newton and Wilson [1] have demonstrated a progressive and marked decrease in nitrogen fixation by red clover plants when they are darkened. Virtanen [2] has reported the conversion of leghaemoglobin to a green pigment in the nodules of plants kept in the dark, and found that such a conversion is connected with the inactivation of the nitrogen fixing system. The present experiments show that there is a striking and rapid decay in the nitrogen fixing capacity of nodules on darkened plants.

Torstai peas were planted in a sand substrate on June 30, were inoculated with *Rhizobium leguminosarum* strain H-43, and were furnished a nitrogen-free nutrient solution during their period of growth in a greenhouse. The plants, in early flower, were approximately 6 weeks old at the time of the experiments, and they were green, vigorous, and well nodulated. The nodules had red centers indicative of the presence of abundant leghaemoglobin.

At harvest the sand was rinsed from the roots, and the nodules were removed from the roots and placed immediately in a small respirometer vessel. The vessel on a Warburg manometer was evacuated [3], flushed twice with O_2 and reevacuated; a mixture

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of N_2 and O_2 to give a pN_2 of 0.1 atmosphere and a pO_2 of 0.2 atmosphere was added followed by argon to atmospheric pressure. The N_2 contained 60 atom % ^{15}N excess. The process of preparing the nodules and gassing them required from 16 to 33 minutes. The nodules, after being shaken in the respirometer for 2 hours, were ground with a mortar in 1.0 *N* HCl, and centrifuged. The sedimented material was discarded and only the acid-soluble portion of the nodules was analyzed (Table 1).

When the experiment was initiated, some of the pots of peas were placed in a large dark room, and the others remained in the greenhouse where they received unsupplemented summer daylight. The experiment was started at 12 noon on August 9, a bright day. After 24 hours in the dark some of the plants were returned to the greenhouse. August 10 and 11 were dull, rainy days, but the morning of August 12 again was sunny.

Although there was but one sample at the initial harvest, it is notable that on this day, when the plants were exposed to high light intensity, their nodules gave the highest fixation of N_2. Fixation by nodules from the lighted plants was much less at the 8 o'clock harvest August 10 than initially; fixation improved somewhat by noon but decreased again by noon August 11. At noon August 12, a bright day, the nodules maintained approximately the same level of fixation as on the previous day.

One sample from the darkened plants retained considerable nitrogen fixing capacity at 8 o'clock August 10, but the amount of nitrogen fixed by nodules harvested from darkened plants at 12 o'clock was very low. The ability to fix nitrogen continued to decay for the next 2 days. The plants returned to the greenhouse after 24 hours in the dark recovered their nitrogen fixing capacity only partially.

No gross change in the pigment of the nodules from the darkened compared to the illuminated plants was apparent after 24 hours, and only a slight decrease in pigment of the darkened plants occurred in 48 hours. At the final harvest (72 hours) the nodules of the darkened plants remained firm but were distinctly green inside

Table 1. Influence of illuminating and darkening pea plants on fixation of nitrogen by their excised nodules. All data are given as atom% ^{15}N excess in the portion of the ground nodules soluble in 1.0 N HCl.

Time of harvest	Treatment of plants	Atom % ^{15}N excess in nodule extract **
12 : 00, Aug. 9	initial control sample	0.627
8 : 00, » 10	greenhouse light * 20 hours	0.220, 0.213
8 : 00, » 10	darkened 20 hours	0.033, 0.210
12 : 00, » 10	greenhouse light 24 hours	0.417, 0.517, 0.520
12 : 00, » 10	darkened 24 hours	0.032, 0.022, 0.019
12 : 00, Aug. 11	greenhouse light 48 hours	0.389, 0.396
12 : 00, » 11	darkened 48 hours	0.012, 0.022
12 : 00, » 11	darkened 24 hours, returned to greenhouse 24 hours	0.233, 0.279
12 : 00, » 12	greenhouse light 72 hours	0.368, 0.398
12 : 00, » 12	darkened 72 hours	0.005, 0.007
12 : 00, » 12	darkened 24 hours, returned to greenhouse 48 hours	0.203, 0.126

* The normal daylight in the greenhouse was not supplemented with artificial illumination. Nights are still comparatively light about August 10 in Helsinki, but the morning in question was cloudy and rainy why light intensity was low. The poor effectivity of the nodules probably depends on this.

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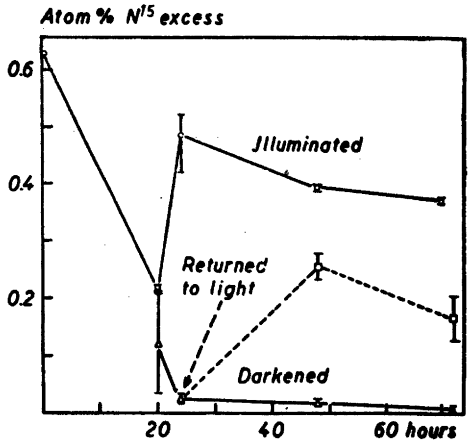

Fig. 1. Influence of illumination of pea plants on fixation by their nodules when exised. Vertical lines define the extreme values of replicate samples.

and exhibited a marked decrease in leghaemoglobin. The nodules from the illuminated plants were indistinguishable from those of the darkened plants which had been returned to light.

It seems most likely that the rapid decay of the nitrogen fixing capacity of nodules from plants kept in the dark for 24 h for the most part arises from depletion (and their rapid recovery in the light from renewal) of substrates furnished normally by the photosynthesizing plant, but partly also from the decrease in leghaemoglobin in the nodules. Virtanen [2] has shown that the oxydation of this pigment to a green one checks N_2-fixation, and a recent investigation by Virtanen and Berg has shown that a smaller part of leghaemoglobin is destroyed even during 24 h in darkness. Thus it can be understood that the transfer of plants to light causes a partial recovery of the effectivity of the root nodules, but far from the whole of it.

Whatever the basis for the observed response may be, the data clearly show that the nodules from pea plants kept in the dark have far less capacity for fixing N_2 than nodules from illuminated plants, and that the lost activity can be partially recovered by returning the plants to the light. It would be of interest to employ the isotopic method to determine in greater detail the rate of decay and recovery of nitrogen fixing capacity, the time required before the injury to the nitrogen fixing capacity becomes irreversible, and the influence of light intensity on the fixation process.

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Estimation of Keto Acids in Plants

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In the study of keto acids the paper chromatography of their 2,4-dinitrophenylhydrazones has been used. As the hydrazones of keto acids form multiple spots on the chromatogram, thus making the identification of the spots uncertain, unknown keto acids are difficult to discover. In order to elucidate unknown acid hydrazones in urine, Kulonen [1] reduced the hydrazones to the corresponding amino acids with aluminium amalgam according to the old method reported by Fischer [2], and investigated the amino acids by paper chromatography. Later Towers, Thompson, and Steward [3] used a similar method, the reduction being made catalytically. We have also developed a modification of the procedure which has already given some interesting results regarding keto acids in plants [4,5]. An account of it is given in the present paper. In our method the reduction is achieved by tin in an alcoholic hydrochloric acid solution, the yield of hydrogenolysis being comparatively good. The purification of the acid hydrazones obtained from the plant material was made according to Virtanen *et al.*[6]

Procedure. 1. *Preparation of the plant extract.* The plant material (usually about 100 g fresh wt.) was homogenized 3 times in a