and slowed reduction at M/1,000. Sodium malonate, sodium azide, 2,4-dinitrophenol, sodium fluoride, and iodoacetic acid merely slowed reduction at M/100 final concentration. Undecylenic acid, a fungicidal agent, stopped reduction completely. From this lack of specificity for inhibitors it would seem that a number of reducing enzymes acting on materials inside the cell can reduce the dye. Aeration by shaking retarded reduction, perhaps because it raised the redox potential too high (over −0.08 v) or because oxygen competed with the TTC.

Experiments on the reduction of TTC by *Penicillium chrysogenum* have added proof to the theory that penicillin is formed by a relatively inactive mold. Apparently a number of reductases can reduce the dye to the colored formazan. TTC promises to be an interesting and useful reagent in studies on cellular physiology.

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Existence of a Tryptophan-Niacin Relationship in Corn

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The role of tryptophan as a precursor of niacin (nicotinic acid) and its metabolites, especially in the presence of adequate amounts of vitamin B_6, has been demonstrated by recent investigations with mammals (*3, 5–7*). It has also been shown that tryptophan serves as a niacin precursor in the fungus *Neurospora* by way of kynurenine and 3-hydroxy anthranilic acid (*2, 4*). On the other hand, neither tryptophan nor any of its intermediates indicated in *Neurospora* seems to affect niacin production in *Lactobacillus arabinosus* and certain other microorganisms (*10*). It has also been reported that the tryptophan-niacin relationship does not exist in the case of bean seedlings grown without their cotyledons on synthetic media (*9*). The present study, however, indicates that a tryptophan-niacin relationship, like that found for certain mammals and *Neurospora*, may exist in a representative higher plant. Experiments in this laboratory have demonstrated that the addition of L-tryptophan to the nutrient medium results in a significantly increased niacin content in mature excised corn embryos grown in sterile culture.

Seeds of a genetically high-niacin corn (Tennessee Inbred 13)[2] were sterilized with $HgCl_2$ and soaked in water overnight. The embryos (including scutella) were removed intact by sterile excision and transferred to individual Erlenmeyer flasks containing a liquid medium consisting of mineral salts, sucrose, and certain vitamins. Two sets of experiments were conducted.

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[2] This seed was kindly supplied by Mr. F. D. Richey of the U. S. Department of Agriculture.

In experiment I various combination concentrations of L-tryptophan and vitamin B_6 (equimolar quantities of pyridoxine, pyridoxamine, and pyridoxal) were added to each 10 ml (per flask) of the basal nutrient solution. The L-tryptophan and B_6 were previously sterilized by bacteriological filtration. The embryos were grown in the dark at 25° C for 10 days and assayed for niacin by the microbiological method employing *Lactobacillus arabinosus* (*1*). Experiment I reveals (Table 1) niacin synthesis in excess of that of the controls to be a direct function of L-tryptophan supply and to be independent of the concentration of vitamin B_6 employed. On a per-plant basis niacin was increased as much as 64% over the controls when 1,000 μg of L-tryptophan, with various concentrations of B_6, were added to the nutrient solution.

TABLE 1

NIACIN CONTENT OF EXCISED CORN EMBRYOS GROWN FOR 10 DAYS IN DARK WITH VARIOUS CONCENTRATION COMBINATIONS OF L-TRYPTOPHAN AND VITAMIN B_6

Experiment I

Treatment (per embryo)	Number of embryos	Average μg niacin per seedling	μg Niacin per gm dry wt
Control	7	5.07	55.8
100 μg L-tryptophan + 100 μg vitamin B_6	8	6.24	62.7
100 μg L-tryptophan + 50 μg vitamin B_6	6	5.78	60.9
100 μg L-tryptophan + 10 μg vitamin B_6	6	6.32	62.3
Mean		6.11 ± 0.17*	62.0 ± 0.6*
500 μg L-tryptophan + 100 μg vitamin B_6	8	7.44	73.7
500 μg L-tryptophan + 50 μg vitamin B_6	4	6.36	73.1
500 μg L-tryptophan + 10 μg vitamin B_6	9	6.54	70.4
Mean		6.78 ± 0.33*	72.4 ± 1.0*
1000 μg L-tryptophan + 100 μg vitamin B_6	8	8.71	85.7
1000 μg L-tryptophan + 50 μg vitamin B_6	8	8.55	96.3
1000 μg L-tryptophan + 10 μg vitamin B_6	9	7.79	86.0
Mean		8.35 ± 0.29*	89.3 ± 3.5*

* Standard error.

In experiment II the effects on niacin synthesis of L-tryptophan and vitamin B_6 individually, as well as in combination, were studied. The data (Table 2) show that the addition of 1,000 μg of L-tryptophan, singly or in combination with vitamin B_6, resulted in a 35% to 42% increase in niacin synthesis over that of the controls on both a per-plant and a dry weight percentage basis. Statistical examination of these data by use of the *t* test and by analysis of variance (multiple classification) (*8*) show the increase to be highly significant ($P < 1\%$). However, the use of L-tryptophan and vitamin B_6 in combination resulted in only a 7% to 9% increase in niacin production

above that obtained by using L-tryptophan alone. This increment is not statistically significant. Nor did the addition of vitamin B_6 alone significantly increase niacin synthesis over that of the controls as checked by the same statistical procedure. The increment was only 3% to 5%. The quantitative differences in the responses of

TABLE 2

NIACIN CONTENT* OF EXCISED CORN EMBRYOS GROWN FOR 10 DAYS IN DARK WITH AND WITHOUT L-TRYPTOPHAN AND VITAMIN B_6

Experiment II

Treatment (per embryo)	Number of embryos	Average µg niacin per seedling	Average µg niacin per gm dry wt
Control	45	5.22 ± 0.11†	63.4 ± 0.1†
53 µg vitamin B_6	45	5.38 ± 0.16	66.3 ± 1.0
1000 µg L-tryptophan	35	6.60 ± 0.28	85.6 ± 1.5
53 µg vitamin B_6 plus 1000 µg L-tryptophan	40	7.06 ± 0.01	90.1 ± 1.4

* Each niacin value represents the mean of 5 replicated treatments, each treatment involving 7–9 seedlings.

† Standard error.

embryos in the two experiments were probably due to the use of seeds from two different crops of the same genetic line.

Niacin assays of the nutrient solutions in which the embryos had been grown indicate that a negligible amount of niacin (< 0.1 µg per plant) was lost by the embryos to the nutrient medium.

It would appear from these experiments with excised embryos that a tryptophan-niacin relationship exists in corn, and that it is independent of added vitamin B_6. Clarification of the role of tryptophan in this relationship, as well as in the normal metabolism of intact corn plants, will be a subject for future investigation. Such information will have added importance in view of the characteristically low tryptophan content of corn.[3]

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[3] Appreciation is expressed to Prof. R F. Dawson of Columbia University for his interest and helpful suggestions.

A New Method of Freezing Eggs in the Shell and Its Possibilities for Further Application on Freezing Foods in General

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Present methods of preserving eggs by freezing require that the eggs be broken out of their shells, stirred to break the membranes, and stabilized by the addition of some material such as salt or sugar (*9*). The use of this product is limited to bakeries, confectioneries, and similar commercial establishments.

Up to the present time, eggs in the shell have been preserved only at temperatures above the freezing point of water. Ordinary cold storage, a combination of cold storage and gas storage (*7*), and other more or less empirical procedures (*8*) are commonly used. All these methods possess certain disadvantages including displacement of the yolk, weakening of the vitelline membrane, considerable loss of weight, acquisition of off-odors and flavors during storage, and various other minor changes.

A method has been developed in this laboratory for freezing eggs without cracking the shell. This minimizes the above undesirable changes, and the eggs are suitable for home use. Furthermore, the resistance of the embryo to freezing temperatures is increased, as shown by hatchability tests now in progress.

The beginning of the process goes back to 1937, when the author was working on the freezing of mushrooms at the Low Temperature Institute, Cambridge, England. There the idea came to him that if the amount of water corresponding to the expansion of the ice formed by freezing were removed uniformly from the tissues, it would probably prevent the disruption of the cells and obviate blanching. The preliminary tests at Cambridge indicated that this idea, despite the fact that the amount of water to be removed from the mushroom was found to be much more than the amount anticipated, was not without merit. The work was continued with more or less successful results on various other products in the laboratory at the Food Research Experiment Station, Athens, Greece, where the author was in charge until 1945. Meanwhile, in 1939, he obtained a Greek patent (*4*) on a process of freezing foods following partial dehydration which actually is the first original and theoretically established process of dehydrofreezing. The work was interrupted by the war, but resumed in Louisiana in 1946.

Since it appeared that expansion of the ice formed from the water of the tissues was the primary cause of most of the major changes occurring during freezing, a study was undertaken of the mechanism of these changes by associating them with the drip (the liquid exuded from the product during thawing). Adequate methods of measuring the drip have been developed (*5, 6*), and and by their use it has been possible to accumulate a multitude of data, to be published later, corroborating