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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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

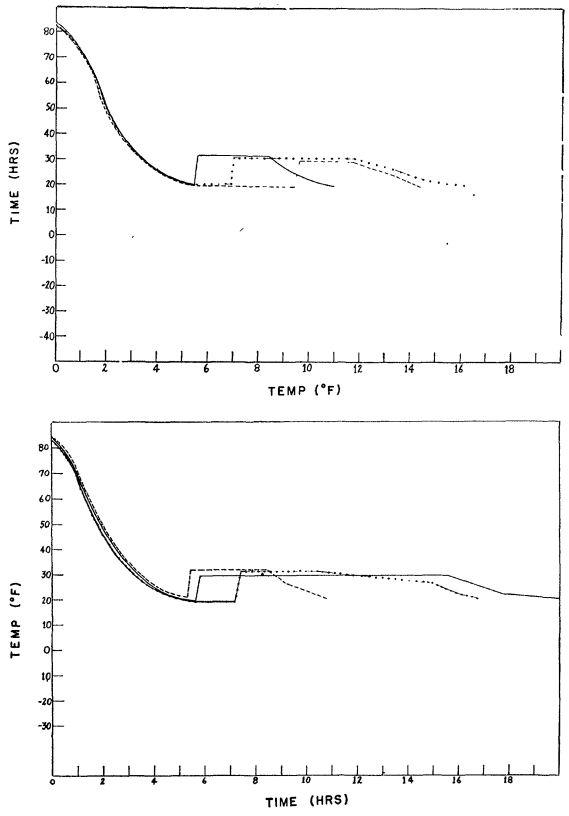
the validity of the assumption that expansion is the primary factor involved in the changes which occur during freezing. One of the controlling factors in the process of expansion during freezing is the relationship of bound water to free water, which determines also the amount of drip. Dehydration affects this relationship as well as drip, though to a lesser extent in the freezing of eggs than in the case of other products.

In the case of eggs the problem was to reduce the water content to the extent needed to provide space for the expansion of the ice formed by freezing. The necessary decrease in weight based on the water content of the egg and its water-ice curve (*3*) was estimated to be 6.1%. The actual experiment showed, however, that elimination of a smaller amount (2–3%) was sufficient for freezing eggs under vacuum without cracking. After finding that the treatment does not greatly change the bound water (the amount of which is rather small) of the egg whites and yolk (Table 1), the effect of a vacuum upon the properties of the egg whites and yolk was examined, since vacuum has been used together with dehydrating agents for the prefreezing treatment. Determinations of the expansion of the egg whites and yolk before and after treatment under vacuum, as well as of their rate of

TABLE 1

AVERAGE VALUES OF CONSTANT PROPERTIES OF EGG WHITES AND YOLK BEFORE AND AFTER TREATMENT (to a Loss of 8% Wt)

	Before treatment			After treatment		
	Liquid	Solid	Yolk	Liquid	Solid	Yolk
Percentage of liquid and solid whites	41	59	.....	49	51	.....
Refractometer reading	14.63	14.61	48.38	17.17	16.76	48.53
pH	8.32	8.24	6.09	8.89	8.99	6.07
Redox potential millivolts at pH 7.0	+158	+156	+116	+139	+133	+95
Freezing point	−0.47	−0.52	−0.58	−0.78	−0.58	−0.60
Expansion by freezing at 20° F	7.20	7.50	2.5	4.85	5.20	2.40
Bound water	3.22	3.39	5.83	3.45	3.66	6.095



FIGS. 1–2. Rates of freezing of the yolk (- - - - -), thick whites (————), and (.....) thin whites of untreated (top) and treated (bottom) eggs.

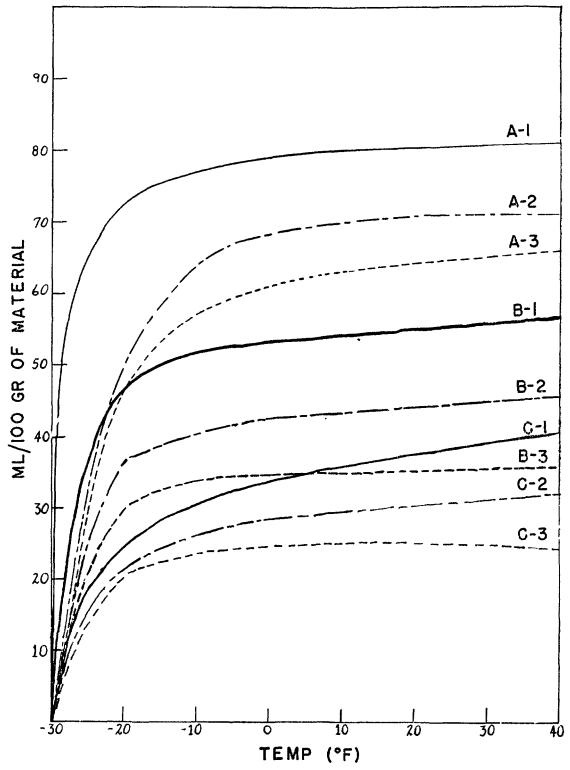


FIG. 3. Expansion of the white and yolk of the egg. A—white: 1, untreated; 2, treated; 3, treated and frozen under vacuum. B—whole eggs: 1, untreated; 2, treated; 3, treated and frozen under vacuum. C—yolks: 1, untreated; 2, treated; 3, treated and frozen under vacuum.

freezing under vacuum, proved tedious until we were able to devise methods which gave reproducible results. The details of these methods will be published later.

The results appearing in Table 1 and graphically in Figs. 1–3 show that freezing treated eggs under vacuum decreases considerably their expansion during freezing and reverses the rate of freezing in the various parts of the egg content when freezing takes place at about 20° F. In the fresh, untreated eggs the white freezes first and creates a solid wall around the yolk, so that when the latter freezes there is no room for expansion and the egg cracks. When the eggs are treated and frozen under vacuum, the yolk freezes first and pushes the white toward the shell so that all the room available in the air space is used during the expansion of the white. This, of course, allows better utilization of the space available within the egg. Removal of the gas from the yolk, on the one hand, and lowering of the freezing point of the white by the treatment, on the other, seem the probable explanation of this surprising result. A contributing factor also is the small decrease in volume of the thick white during the freezing of the treated eggs through a partial breaking down of its mucin content, due probably to the change of pH under the reduced pressure of CO_2 (*1*), a fact which is associated with a change in volume. In one particular case, where the change of thick white to liquid was about 50%, the change in volume was about 3%. All these factors working together help to secure a better economy of space within the egg during freezing and reduce the necessary treatment to a minimum. Under these conditions the treatment does not affect the quality of the eggs at all; on the contrary, the whipping quality of the white is somewhat improved. The loss of weight during storage at 20° F and 85% relative humidity was, in our experiments, 0.15% per month, in comparison with a 0.23% loss obtained during the storage at about 32° F. This loss would be still less if the humidity of the room were kept higher. Eggs kept in an undercooled condition at 23° F for 7 months lost 1.8–2.9% (*10*), and if the theoretical formula developed by Greenlee (*2*) is valid, the calculated loss at storage temperatures below 28° F is zero.

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Reversal to Penicillin Sensitivity in a Cysteine-requiring Mutant of *Salmonella*[1]

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The action of penicillin in producing bacteriostatic and bactericidal effects is believed to be due to chemical interactions with essential SH and perhaps NH_2 groups in the medium (Bailey and Cavalitto, *1*), and so a metabolic block is produced which may be irreversible. Additional evidence for the point of view that penicillin is a metabolic antagonist has recently been given by Wilson and du Vigneaud (*7*), who showed that L-penicillamine—but not D-penicillamine—inhibits the growth of young rats when added to a diet which otherwise permits good growth. When aminoethanol and methylated derivatives were added to the L-penicillamine-containing diet the inhibiting effect of the latter was counteracted. More direct evidence of specific metabolic antagonism is given by Gale and Rodwell (*2*), whose experiments with *Staphylococcus* are interpreted as showing that penicillin acts to impair the ability to assimilate glutamic acid. These investigators have studied the nutritional requirements of penicillin-sensitive *Staphylococcus*, and of resistant strains derived from the original culture by training or mutation. It appears that the parental strains cannot synthesize most of the essential nutrilites, but can concentrate within the cell free amino acids, especially glutamic acid, provided they are available in the surrounding medium. The mutant resistant strains have lost the ability to concentrate free glutamic or other amino acids, but have concurrently developed the power to synthesize all these essential nutrilites within the cell from their inorganic constituents. Since the mutant strains are thus not dependent for growth on the assimilatory processes they should be independent of the antagonistic effects of penicillin, as they are.

These studies still leave many questions unanswered. One of the most obvious concerns the status of the Gram-positive organisms like *Bacillus subtilis*, which are heterotrophic, i.e., synthesize all their nutrilites except glucose, yet are still penicillin-sensitive. It is of considerable interest, therefore, to test the penicillin sensitivity of nutritional mutants of other bacteria, particularly those that are originally penicillin-resistant.

We wish to record one such series of tests in a Gram-negative organism, the results of which give excellent correlation with the metabolic antagonism theory of the action of penicillin. We are studying radiation-induced mutations in *Salmonella typhimurium*, a food poisoning pathogen which is heterotrophic and highly resistant to penicillin. A number of different kinds of mutations

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