which the liquid can be subcooled. Alternatively, when the value of T_c is known, the equation can be solved for the interfacial tension, σ.

Consider, for example, the freezing of water, for which the value of T_c is known to be $T_c = -38°$ C. Taking $T_0 = 273°$ K, $T_c = 235°$ K, $\Delta H_v = 80$ cal/cc $= 3.34\ (10)^9$ ergs/cc, the value of the ice-water interfacial tension, σ, determined from equation (5) is

$$\sigma = 32.8 \text{ ergs/cm}^2. \qquad (6)$$

By using this value of σ in equation (1), the rate of nucleation, $\dot{n}$, can be obtained as a function of T:

T° C	$\log_{10} \dot{n}$
0	$-\infty$
−33	−10.89
−34	− 8.33
−35	− 5.98
−36	− 3.82
−37	− 1.84
−38	0
−39	+ 1.70
−40	3.27
−41	4.74
−42	6.10
−43	7.37

It is of interest to note that the rate of nucleation changes by a factor exceeding 10^{18} in a 10° temperature range including T_c as midpoint. T_c, therefore, resembles a critical temperature, in that water cooled to a few degrees above T_c can persist as liquid for many years on account of the small rate of nucleation of ice. However, on lowering the temperature from a few degrees above T_c, the rate of nucleation increases so rapidly that subcooling below T_c is highly improbable.

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The Reduction of 2,3,5-Triphenyltetrazolium Chloride by *Penicillium chrysogenum*[1]

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The use of the tetrazolium salts, especially 2,3,5-triphenyltetrazolium chloride, to indicate cell viability is aparently well established. Triphenyltetrazolium chloride (TTC) has been used to indicate the germinability of seeds (*3*), and as a histological agent and a reagent in rapid penicillin assays with *Staphylococcus aureus* (*4*). Its use as a reagent in physiology depends upon the enzymic reduction of the soluble colorless triphenyltetrazolium salt to an insoluble carmine red formazan. At this laboratory TTC has been used in studies on the physiology of the penicillin-producing strains of *Penicillium chrysogenum.*

Penicillium chrysogenum Q176 was grown in shaken flasks of 2% corn steep solids–2% lactose medium after the manner described by Koffler, *et al.* (*2*). After harvesting, the pellets were washed free of pigments and nutrients, suspended in M/15 phosphate buffer, and torn apart by a 5-sec treatment in a Waring Blendor. After blending, a buffer solution of the dye was added to give a final concentration of 0.5% TTC. The buffer used throughout was at pH 7.2–7.4, because the reduction of the dye was retarded at a lower pH and was virtually stopped at pH 6.

Under these conditions the most active mold cells would reduce the dye to a deep red color in 20 min at 30° C. Table 1 shows the relative ability of *Penicillium chrysogenum* Q176 of different ages to reduce the dye to a colored formazan and gives the penicillin yields at the time of harvest.

TABLE 1

A CORRELATION BETWEEN THE RATE OF TTC REDUCTION AND PENICILLIN YIELDS BY MYCELIUM OF *Penicillium chrysogenum* Q176 AT DIFFERENT AGES

Age of mycelium (Days)	Color	Penicillin yield (Oxford units/ml)
1	deep red	0
2	deep red	0
3	red	0
4	pink-red	0
5	pink-red	42
6	yellow-pink	294
7	yellow-pink	440
8	yellow (color of mycelium)	310

These experiments have been repeated on other penicillin-producing molds with the same results. Thus, if TTC reduction is an indicator of cell viability, it is evident that young nonpenicillin-producing cells (1–3 days old) are much more viable than the older penicillin-producing cells (5–7 days old). In other words, penicillin is formed by the mold when its metabolic state is considerably reduced. That penicillin is formed by the mold when its metabolic state is low or abnormal has been suggested (*1*). These findings with TTC have been checked with the vital stains Nile blue sulfate and neutral red. The cytoplasm of young cells that readily reduced TTC stained deeply and homogeneously, while cells 5 to 7 days old stained unevenly and showed the granules and vacuoles typical of aged cells.

TTC was reduced only inside the cells and the addition of glucose did not change the rate or site of reduction; apparently the endogenous activity of the mycelium was more than sufficient to reduce the dye. The inhibitor KCN inhibited reduction at M/100 final concentration

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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.

[1] Lalor Fellow.

[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