Table 3. Mean percentages of trials per subject on which nonsense and meaningful prerecognition responses were given.

Responses	Method			
	Limits		Random series	
	Mean	S.D.*	Mean	S.D.
Nonsense	25.3	10.8	19.3	6.8
Meaningful	10.1	6.7	10.2	4.1

* S.D., standard deviation.

the frequency of meaningful prerecognition responses and the speed of recognition are similar for the two methods.

Meaningful responses, including incorrect guesses and correct recognitions, depend on the discrimination of stimulus fragments which enable the subject to attempt a reconstruction of the stimulus word. It appears that successive exposures of the same word do not substantially accelerate the discrimination of minimally effective stimulus fragments. At the very least, the influence of cumulative presentations is masked by the effects of exposure duration as such.

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References and Notes

1. For illustrations of this method, see D. H. Howes and R. L. Solomon, *J. Exptl. Psychol.* **41**, 401 (1951); R. L. Solomon and L. Postman, *J. Exptl. Psychol.* **43**, 195 (1952).
2. Examples may be found in G. A. Miller, J. S. Bruner, L. Postman, *J. Gen. Psychol.* 50, 129 (1954); L. Postman and M. R. Rosenzweig, *Am. J. Psychol.* **69**, 209 (1956).
3. E. L. Thorndike and I. Lorge, *The Teachers' Wordbook of 30,000 Words* (Bureau of Publications, Teachers' College, Columbia University, New York, 1944). Averages of the L and S counts were used in determining the frequencies of usage.
4. E. McGinnies, P. B. Comer, O. L. Lacy, *J. Exptl. Psychol.* **44**, 65 (1952).
5. F. Wilcoxon, *Some Rapid Approximate Statistical Procedures* (American Cyanamid Co., New York, 1949).

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Mechanism of Freezing in (Plant or Animal?) Living Cells and Tissues

The recent paper by Meryman (*1*) includes a treatment of the physical principles of ice-crystal growth that fills a real need for all biologists. But his treatment of "Freezing in cellular biological systems" almost completely ignores the vast amount of work that has been done on plants (*2*). As a result, much of what he says certainly does not hold true for plant cells. The following are a few cases in point:

1) "The lethal factor . . . is the exceedingly high concentration of electrolyte resulting from the removal of water. . . ." This theory was proposed 50 years ago to explain frost injury to plants. Some 6 years later it was completely disproved by Maximov, who showed that cells that are normally killed at −5°C survive −20°C if they are frozen in nonpenetrating and nontoxic solutions. Many other lines of evidence oppose the theory in the case of plant cells (*2*).

2) "If the specimen survives this far [−10°C], further decrease in temperature causes no further change in the degree of dehydration. . . ." Direct measurements by Scholander and coworkers (*2*) have shown continuous and progressive increases in ice formation both in animal and plant tissues down to about −30°C.

3) "Whether this [intracellular freezing of dead cells] is simply a reflection of loss of viability and membrane permeability . . . has not been experimentally investigated." It has been experimentally investigated by several workers, including Chambers and Hale, whom Meryman cited. The evidence indicates that membrane permeability is the cause of the intracellular freezing.

4) "It is nevertheless a fact that crystallization is wholly or predominantly extracellular until rather rapid rates of freezing are obtained. . . ." The speed of freezing that results in the formation of intracellular ice varies markedly among plants, particularly when hardy and nonhardy plants are compared. To define rapid and slow freezing on the basis of the rate needed to induce intra- or extracellular freezing (as Meryman does) would imply that rapid freezing in some plants is slower than slow freezing in others.

5) "In addition to the lethal potential of intracellular crystal growth, rapid freezing also creates a dehydration with the same potential for denaturation. . . ." Injury from intracellular freezing occurs at much higher temperatures (and therefore milder dehydrations) in hardy plants than does injury from extracellular freezing. Furthermore, in nearly all cases among the vast number that have been reported, intracellular freezing injury has occurred practically instantaneously. Extracellular freezing injury, on the other hand, as well as other kinds of dehydration injury (for example, plasmolysis injury) increases with the time of exposure to it (*2*). Finally, no plant cells have yet been discovered that are able to survive intraprotoplasmic freezing at moderate temperatures, although some are able to survive much greater dehydration than others. Consequently, dehydration can play no part in the injury produced.

6) "The rapidity with which destructive ice crystals can grow in the solid state renders the thawing procedure equally, if not more, demanding than the freezing procedure." This may be true of the extremely rapid and intense freezing that occurs when small pieces of tissue are plunged into liquid air, but it does not apply to more moderate freezing (for example, at −10°C) that is still rapid enough to produce intracellular ice formation in plant cells. In such cases (see previous paragraphs) the cells are always killed, regardless of the speed or nature of the thawing process.

7) "The addition of glycerine . . . limits the degree of dehydration produced." It is very easy to show that this is not true in the case of plant cells (*2*). When glycerine is allowed to penetrate the cells, the best that can be obtained is an ability to withstand temperatures 2 or 3 degrees lower. When the cells are frozen immediately in the glycerine solution before appreciable penetration has occurred (or in other solutions that do not penetrate) they can be made to survive a temperature 15°C lower. Yet the dehydration in the latter case is much greater.

There are perhaps three main reasons for expecting differences in the freezing behavior of plant cells and of the kind of animal cells that Meryman is mostly concerned with: (i) the (mainly cellulose) cell wall surrounding plant cells, (ii) the bathing fluid around the animal cells, and (iii) the large vacuole in each mature cell, at least of higher plants. Whether or not there are really major differences between the mechanisms of freezing in plant and animal cells, I do not know. But it seems obvious that a better understanding of the latter would be sure to result from better acquaintance with the work on plants (and vice versa). I would therefore like to suggest a greater exchange of reprints between the animal and plant scientists in this field as well as in others.

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References

1. H. T. Meryman, *Science* **124**, 515 (1956).
2. J. Levitt, *The Hardiness of Plants* (Academic Press, New York, 1956).

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Levitt's observation that the article, "Mechanics of freezing in living cells and tissues," is primarily concerned with animal material is quite correct, and possibly the title should have so indicated. However, although it would, in retrospect, have been advisable to include more allusions to plant material, detailed discussions of freezing in specific tissues was, as stated in the introduction, not the

primary purpose of the article. One of the major problems in attempting to unravel the mechanism of freezing in either the botanical or zoological areas has been the confusing variability in response to freezing by different individual cells and tissues. This variability has often obscured the underlying physical phenomena that are common to all. It was the objective of the article to discuss the basic phenomena and to indicate, using soft tissue cells as a principal example, some of the responses to be expected.

The quotations which Levitt has selected serve quite well to demonstrate some of the specific differences in the responses of plant cells to freezing, and it is indeed unfortunate that the existence of such dissimilarity was not made clear in the original article. However, these specific differences, which are concerned primarily with survival, must not be permitted to obscure the relevance of the underlying general mechanics. To imply, as Levitt does in paragraphs 5, 6, and 7, that dehydration from rapid freezing, recrystallization during thawing, or water binding by glycerine do not apply to plant cells because the cells will have already been killed is to subjugate general principles to a special application. A semirigid cell wall and an essentially water-filled vacuole will, of course, produce a different response to ice crystal formation from that of muscle or liver, just as these tissue responses differ from those of bone or tendon. Despite this variability, crystal growth, dehydration, recrystallization, and all the other physical events attending freezing and thawing inevitably take place regardless of the point in the sequence of events at which the cell is killed. Water binding by glycerine always reduces the dehydration from freezing, and whether or not the cell derives any benefit thereby has no influence on the validity of this fact. It was my experience that a study of the myriad responses of different cells to freezing led to a most confusing assemblage of inconsistent conclusions until the fundamental mechanics of the process had been outlined and a physical basis for interpretation had been provided. It was primarily to provide a hypothesis for this physical basis from which others could interpret their own special applications that this article was written.

Levitt also touches on one point that surely troubles many scientists in many fields: the practical unavailability of knowledge of common value derived by workers in other disciplines. The exchange of reprints cannot, in itself, be an answer, for this presupposes a knowledge of the other man or his work. Much of the responsibility must lie with the individual investigator to evaluate those aspects of his own work that may be of general interest beyond his immediate field and to present them, as much as possible out of their specialized context, in a journal of not too highly specialized nature. The bias of his interests will inevitably show through but, it is hoped, not so strongly that it frightens away the potential beneficiary.

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Carbon-14 Tetrachloride Produced by Neutron Irradiation of Aniline- and Pyridine-CCl_4 Solutions

The recoil method of synthesis for C^{14}-labeled compounds involving neutron irradiation of nitrogen-containing compounds dissolved in other solvents or in a pure state has been discussed by a number of investigators (*1, 2*). This method of synthesis depends on the high recoil energy (approximately 40,000 ev) of the C^{14} atom that is acquired following the $N^{14}(n,p)C^{14}$ reaction. The recoil energy of the C^{14} atom produced by the emission of the proton is dissipated by collisions with neighboring molecules, which often result in bond breaking of the struck molecules. In the recombination of these molecular fragments, the C^{14} atom may be included, and thus a C^{14}-labeled molecule is produced. This study (*3*) reports on the production of $C^{14}Cl_4$ from the neutron irradiation of solutions of aniline ($C_6H_5NH_2$) and pyridine (C_5H_5N) in CCl_4. Although the carbon tetrachloride produced was of a low specific activity, the simplicity of the procedure recommends it over chemical synthesis.

Five milliliters of a 10 mole percent solution of pyridine in carbon tetrachloride was sealed in a partially evacuated quartz capsule held at Dry Ice temperature. Five milliliters of 10 mole percent of aniline in carbon tetrachloride was prepared in a similar way. The samples were irradiated for 1 month in the Oak Ridge graphite reactor at an average neutron flux of 3×10^{11} neutrons cm^{-2} sec^{-1} and gamma ray flux of 5×10^5 r hr^{-1}. The solution of pyridine in CCl_4 following irradiation was a dark liquid with large particles present. When the capsule was opened in a manner previously described (*4*), there appeared to be little or no build-up of pressure in the sample. The aniline-CCl_4 solution appeared to be solid and almost opaque. When it was opened, it was apparent that pressure had built up in the capsule, and the sample became a liquid after release of the pressure. It was similar in appearance to the pyridine-CCl_4 solution with one exception. A tough, dark polymer covered the liquid surface and reacted slowly with added CCl_4 with a hissing noise similar to the sound of a small piece of sodium in water.

Because the neutron irradiation of CCl_4 produces considerable quantities of S^{35}, certain procedures were necessary to obtain pure fractions of CCl_4 and to measure only C^{14} radiations (*5*). After addition of carrier CCl_4 and removal of 1-ml aliquots for determination of total C^{14} originally present, the amines were extracted with three 10-ml washes in 3*N* HCl and one water wash. The CCl_4 layer was then refluxed for 1 hour with a sodium hypobromite solution to oxidize any sulfur-containing compounds, and then the CCl_4 was distilled into three fractions and a residue.

Fifty-microliter samples of the purified CCl_4 fractions and of the original samples were combusted in a Pregl-type combustion tube. The $C^{14}O_2$ was trapped in a standardized NaOH solution and the percentage of carbon recovered was determined by titration procedures. Silver wire present in the combustion tube filling should absorb any oxides of sulfur still present. After transfer to an apparatus described by Comar (*6*), the NaOH solution with the trapped $C^{14}O_2$ was acidified and heated to boiling; the CO_2 was swept out through a mercury seal into an evacuated 250-ml ionization chamber. The disintegration rate as a function of ionization current was then determined with a vibrating reed electrometer .

Table 1 lists the experimental values found for the various fractions and the originals. About 31 percent of the total C^{14} activity measured was found in CCl_4 from both samples. This would tend to corroborate the earlier report (*2*) that the nature of the carbon hot-atom reactions is a characteristic of the solvent. The residue left after distillation was quite active, although no quantitative

Table 1. Carbon-14 activity of the purified CCl_4 fractions and of the original solution after the addition of carrier CCl_4.

Fraction	No. samples (No.)	Boiling point range (°C)	Activity (disintegrations/sec 50 µl)
	Pyridine-CCl_4 solution		
1	2	70–74	9.6
2	2	74–75	10.5
3	3	75	10.4
Original	2		32.5
	Aniline-CCl_4 solution		
1	3	45–72	7.4
2	2	72–75	6.7
3	2	75	4.5
Original	4		20.0