not hibernate. The behavior of animals that did not hibernate may be related to the effects of aversive conditions on memory, reported by Weiskrantz (*7*). Clinical reports of retrograde amnesia indicate that the memory for the most recent experiences is the most susceptible to disruption. Apparently the cold environment acted as a stressful situation to awake animals; this could explain the retrograde effects.

The interference theory of forgetting suggests that material is lost to memory only when it is displaced by some other material. The mere passage of time between initial learning and recall does not cause forgetting. All animals were in the cold for the same periods. Those animals that hibernated blocked out the effects of interfering experiences, whereas those animals that remained alert during the cold-exposure periods were subjected to stimuli that may have interfered with encoding of the original learning task.

A variety of brain electrical activity patterns has been recorded on animals in hibernation (*8*). Chatfield and Lyman (*9*) showed three types of activity in animals aroused from hibernation: (i) bursts of activity at a frequency of 17 sec^{-1}, (ii) bursts of spikes, and (iii) continuous waves of activity. Others reported low-voltage high-frequency desynchronized cortical activity without arousal.

Pengelley and Fisher (*10*) reported that ground squirrels become habituated to stimuli while in hibernation. These experiments indicate that the nervous system of hibernators is capable of functioning at low body temperatures (1°C), at least to the extent that habituated response to a stimulus can be made. It is clear the animal is maintaining an integrated control.

During hibernation the cortex may be nonfunctional. However, cortical silence associated with lowered body temperature does not pose a threat to the survival of the animal because important thermoregulatory centers are subcortical. Lyman and O'Brien (*11*) concluded that peripheral temperature receptors in *C. tridecemlineatus* and *C. lateralis* are not involved in temperature regulation during hibernation, and that accurate sensing of declines in brain temperature allows the hibernator to arouse from lethal cold.

Animals that hibernated had better retention scores. The most reasonable interpretation of this result is that hibernation eliminates or greatly reduces cortical activation and thereby protects memory traces from being eliminated. The significance of memory retention in the natural life history and evolution of the hibernator can now be surmised. Among hibernators, the animals that do not hibernate or hibernate only briefly during a winter may lose behavior patterns that are essential to the survival of the species.

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References and Notes

1. J. L. McGaugh and R. G. Sawson, *Behav. Sci.* **16**, 45 (1971).
2. D. J. Lewis, *Psychol. Rev.* **176**, 461 (1969).
3. R. P. Kesner and J. A. D'Andrea, *Physiol. Behav.* **7**, 73 (1971).
4. N. Mrosovsky, *J. Comp. Physiol. Psychol.* **56**, 811 (1963); J. A. Panuska and V. P. Popovic, *J. Appl. Physiol.* **20**, 1275 (1965).
5. N. Mrosovsky, *Anim. Behav.* **12**, 454 (1964).
6. M. C. McNamara, thesis, University of New Mexico (1972).
7. L. Weiskrantz, in *Amnesia*, C. W. M. Whitty and O. L. Zangwill, Eds. (Butterworth, Washington, D.C., 1966), pp. 320–324.
8. L. J. T. Mihailovic, in *Hibernation and Hypothermia Perspectives and Challenges*, F. E. South, J. D. Hannon, J. R. Willis, E. T. Pengelley, Eds. (Elsevier, New York, 1972), pp. 487–534.
9. P. O. Chatfield and C. P. Lyman, *Electroencephalogr. Clin. Neurophysiol.* **6**, 403 (1954).
10. E. T. Pengelley and K. C. Fisher, *J. Mammal.* **49**, 561 (1967).
11. C. P. Lyman and R. O'Brien, *Amer. J. Physiol.* **222**, 864 (1972).
12. Supported in part by NSF grant GB 5339.
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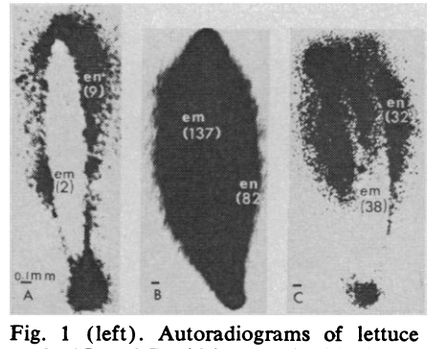
Dichloromethane and Lettuce Seed Germination

Meyer and Mayer (*1*) have proposed the use of dichloromethane (DCM) for introducing substances into dry seeds. If the potential of this method were realized, as pointed out by the authors, it could be useful in overcoming practical problems of seed storage. I therefore investigated the effectiveness of DCM for introducing chemicals into dry seeds. The lack of toxicity of DCM to dry whole seeds, was confirmed; however, under my experimental conditions DCM was not entirely successful in introducing coumarin into the embryo of dry lettuce seeds.

Treating lettuce seeds with DCM (*2*) for 24 hours had little effect on subsequent germination (Table 1). When seeds were treated with 5 or 10 m*M* coumarin in DCM, germination was inhibited, but not as severely as previously reported. Inhibition was reversed by rinsing the seeds for 20 seconds in DCM, an indication that the



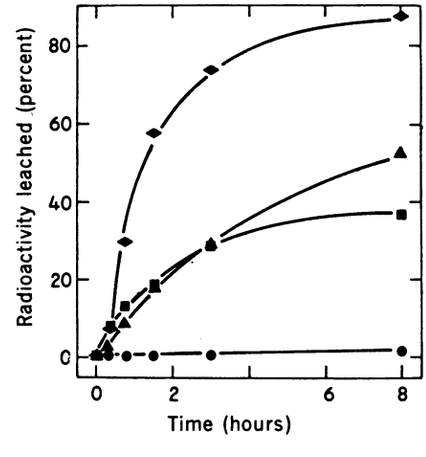


Fig. 1 (left). Autoradiograms of lettuce seeds (Grand Rapids) treated with 5 m*M* [3-^{14}C]coumarin (0.5 μc/ml). (A) Whole seeds treated 24 hours with [^{14}C]coumarin in dichloromethane; (B) whole seeds treated 18 hours with [^{14}C]coumarin in water; and (C) scarified seeds treated with [^{14}C]coumarin in dichloromethane. Treated seeds were cut transversely into three sections with each section mounted on two-faced tape, and the cut surface placed in direct contact with x-ray film for 13 days. Numbers in parentheses represent nanomoles of coumarin associated with the embryo or endosperm complex from ten seeds treated with [^{14}C]coumarin; *em*, embryo; *en*, pericarp-integument-endosperm. Fig. 2 (right). Radioactive material leached from lettuce seeds (Grand Rapids) containing [3-^{14}C]coumarin; whole (●) or cut (■) seeds in dichloromethane and whole (▲) or cut (◆) seeds in water. Seeds imbibed aqueous 5 m*M* [3-^{14}C]coumarin (0.5 μc/ml) for 18 hours, and were then rinsed, blotted, and dried in air. Then ten whole or longitudinally cut seeds were placed in 1 ml of distilled water or dichloromethane. Rate of leaching was determined by removing and replacing each solvent after 0.05, 0.17, 0.67, 1.67, 3, and 8 hours. Radioactivity in the leachate and that remaining in the seeds after 8 hours was determined.

Table 1. Average germination of four cultivars of lettuce seeds (Great Lakes, Grand Rapids, Golden State, and Vanguard) treated for 24 hours with dichloromethane containing coumarin (5 or 10 m*M*). After treatment, seeds were rinsed zero to two times for 20 seconds with DCM, dried in air for at least 12 hours, and then allowed to germinate for 24 hours at 20°C in the dark. At $P < .05$, least significant difference is 14; at $P < .01$, least significant difference is 18.

Treatment	Germination (%)
None	93
Dichloromethane	89
Dichloromethane + coumarin	60
Dichloromethane + coumarin + one rinse	84
Dichloromethane + coumarin + two rinses	86

coumarin was outside the seed, rather than in the embryo (*3*).

Experiments with [3-^{14}C]coumarin in DCM (Fig. 1A) showed that 83 percent of the coumarin associated with the seed after it was rinsed with DCM was in the "pericarp-integument-endosperm complex" surrounding the embryo (*4*). Furthermore, the autoradiograph of seeds treated with coumarin and DCM shows that the radioactivity is associated with the pericarp-integument-endosperm complex, not with the embryo. This contrasts with the uniform distribution of radioactivity in lettuce seeds that have imbibed water containing [^{14}C]coumarin (Fig. 1B).

Coumarin in DCM may fail to reach the embryo because DCM does not penetrate the pericarp-integument-endosperm complex. To test this, whole and scarified lettuce seeds were treated with DCM (*5*). While scarification itself has little or no effect on germination, treating scarified seeds with DCM reduced germinability. In scarified seeds, however, coumarin in DCM did reach the embryo (Fig. 1C), but the amount and distribution were different than they were with coumarin in water. Apparently, the endosperm complex acts as a barrier to some organic solvents, as well as to inorganic ions and amino acids (*6*).

Leaching experiments, with dry lettuce seeds that had previously imbibed aqueous 5 m*M* [^{14}C]coumarin, gave further evidence that DCM does not get into whole seeds (Fig. 2). The low level of radioactive material leached from whole seeds, containing radioactive coumarin, into DCM indicates that DCM had not penetrated the seeds. This conclusion is based on tests showing that radioactive material was leached from cut seeds by DCM, and from cut and whole seeds by water. These data show that coumarin can be leached from seeds, if the solvent gets in.

Dichloromethane does not appear to be a useful tool for putting chemicals into lettuce seed embryos because of its poor penetration through the endosperm complex.

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References and Notes

1. H. Meyer and A. M. Mayer, *Science* **171**, 583 (1971).
2. Results were similar when the dichloromethane was either redistilled over Na_2SO_4 or contained 0.01 to 0.02 percent water.
3. H. Meyer germinated seeds at 26°C (personal communication). I could also show inhibition at 26°C. Lettuce seeds are more sensitive to coumarin at this temperature than at 20°C [M. Evanari, *Palestine J. Bot. Jerusalem Ser.* **5**, 138 (1952)].
4. H. A. Borthwick and W. W. Robbins, *Hilgardia* **3**, 275 (1928).
5. Germination of scarified seeds (distal tip of seed removed) either untreated or treated for 24 hours in dichloromethane occurred in 88 percent or 18 percent of the seeds, respectively.
6. A. H. Haber and N. E. Tolbert, *Plant Physiol.* **34**, 376 (1959); S. Klein, M. Negbi, A. Witztum, L. Rothberg, *New Phytol.* **70**, 143 (1971).
7. I thank G. Carpenter for his help in preparing autoradiograms.

3 July 1972

We have confirmed the findings of Meyer and Mayer (*1*) that lettuce seeds can be stored in dichloromethane, acetone, or ethanol for extended periods (of at least several months) without loss of viability, as shown by germination tests after evaporation of the solvents in a vacuum (Table 1). We also found that appreciable amounts of water (for example, 8 percent in ethanol) in the solvent rapidly causes loss of the viability. We concluded, however, that the survival of the lettuce seeds under these pure solvents was not the result of any capacity of the embryo to withstand the solvents. On the contrary, the survival resulted from the inability of the solvents to reach the embryo, owing to the impermeability of the endosperm. Our conclusion was based on the following findings: (i) half seeds, cut so as to remove the endosperm as a barrier between the external solution and the embryo, lost viability when immersed in the solvents (Table 1); (ii) cut seeds that were not exposed to the solvents retained viability (Table 1); (iii) a variety of organic dyes that were dissolved in the pure solvents failed to penetrate the endosperm of intact seeds (Table 1); (iv) the organic dyes were seen to penetrate to the embryo of intact seeds when the solvents had sufficient amounts of water to cause loss of viability; and (v) the organic dyes dissolved in the pure solvents did reach the embryo of cut seeds (Table 1).

Our results fail to confirm the possibility of using organic solvents to supply test chemicals to embryos in intact lettuce seeds. The apparent effective-

Table 1. Penetration of solvents and their effect on germination of lettuce seeds. Seeds (intact or cut transversely into approximately equal halves) were immersed in solvents (without dyes) for 24, 48, or 72 hours. They were then dried in a vacuum and allowed to germinate for 72 hours in water in a growth chamber with 7700 to 8800 lu/m² of mixed incandescent and fluorescent white light during a photoperiod of 16 hours light (21° ± 1°C) and 8 hours dark (18.5° ± 1°C). Germination percentages of half seeds were determined from the micropylar half, containing the entire embryonic axis. In other experiments, dyes were dissolved in the solvents. After evaporation of the solvent in a vacuum the seeds were examined to see if the dyes had penetrated to the embryo. Symbols: P, dye penetrated to embryo; N, dye did not penetrate to embryo; —, no sample. Data apply to each of the following dyes: Pyronine B, carmine, safranin O, methyl green, and toluidine blue.

Seed response	Intact seeds			Half seeds		
	Time in solvent (hours)			Time in solvent (hours)		
	24	48	72	24	48	72
	No solvent					
Percent germination (vacuum controls)	100	100	100	100	100	100
Uptake of dye (water controls)	—	P	—	—	P	—
	Dichloromethane					
Percent germination	100	100	97.5	57.1	0	0
Uptake of dye	—	N	—	—	P	—
	Acetone					
Percent germination	100	100	100	0	0	0
Uptake of dye	—	N	—	—	P	—
	Ethanol					
Percent germination	100	100	100	100*	92.4*	77.7*
Uptake of dye	—	N	—	—	P	—

* The uptake of ethanol, indicated by visible swelling, proceeded only very slowly from the cut surface of the half seed toward the micropylar end.

ness of a test chemical, for example, coumarin dissolved in acetone or dichloromethane (*1*), might nevertheless be expected. A nonvolatile test chemical would still be retained on surfaces exterior to the embryo after evaporation of the solvent. Subsequent imbibition by the seeds in water would then introduce the test chemical into the embryo just as readily as if the test chemical had originally been in aqueous solution.

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Reference and Note

1. H. Meyer and A. M. Mayer, *Science* **171**, 583 (1971).
2. Oak Ridge National Laboratory is operated by the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

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Triplett and Haber (*1*) and Anderson (*2*) essentially confirm our experimental results (*3*). However, they argue that treatment of whole seeds by organic solvent and coumarin does not result in the inhibitor reaching the embryo. In our original report (*3*), we made no claim with regard to the location of the coumarin that modifies the subsequent germination behavior of the seeds. We accept the view that the bulk of the inhibitor is located in the endosperm complex, as suggested by Anderson (*2*). The endosperm in lettuce seems to control germination behavior to a marked extent (*4*). Moreover, the amount of coumarin required to reach the embryo in order to inhibit germination is exceeding small—1 nmole per seed (*5*). Thus, the results of Anderson do not seem to us to be seriously at variance with our own. The experiments of Triplett and Haber (*1*) do not necessarily prove damage to the embryo by dichloromethane (DCM), as treatment for 24 hours with DCM resulted in germination in 57 percent of the seeds, and they do not describe in any detail what happens to half seeds with regard to their light sensitivity, for example, which is probably reduced or lost. Their use of unstated dyes is difficult to analyze, and is not necessarily a good indicator of what happens in the seed.

The conclusion of both Triplett and Haber (*1*) and of Anderson (*2*) that organic solvents cannot be used to introduce chemicals into lettuce seed embryos may be justified. However, the use of organic solvents to treat entire seeds in order to modify their subsequent germination behavior appears to us to be a perfectly valid one, and not in any way contradicted by the results of Triplett and Haber (*1*) and of Anderson (*2*). The use of an organic solvent in treatment of seeds appears to have been first suggested by Millborrow (*6*).

We now have evidence that radioactive compounds introduced by DCM technique into dry seeds are metabolized normally by the seeds, and are incorporated into protein (*7*). The effect of treatment with organic solvents on other seed species, in which the endosperm problem is less marked—for example, peas, wheat, barley, and so forth—is as yet undetermined.

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References

1. L. Triplett and A. H. Haber, *Science* **179**, 95 (1973).
2. J. D. Anderson, *Science* **179**, 94 (1973).
3. H. Meyer and A. M. Mayer, *Science* **171**, 583 (1971).
4. M. Evenari and G. Neuman, *Bull. Res. Council Israel* **2**, 15 (1952); S. Klein and J. W. Preiss, *Plant Physiol.* **33**, 321 (1958); J. W. Preiss and S. Klein, *ibid.*, p. 326; S. Klein, M. Negbi, A. Witztum, L. Rothberg, *New Phytol.* **70**, 143 (1971).
5. A. M. Mayer, *Physiol. Plant.* **6**, 413 (1953).
6. B. V. Millborrow, *Nature* **199**, 716 (1963).
7. M. Eldon and A. M. Mayer, unpublished observations.

30 October 1972

Nitrosation in the Environment: Can It Occur?

Although the organic chemist can readily demonstrate the production of *N*-nitrosodimethylamine, in high yield, from dimethylamine and nitrite in the chemical laboratory, the course of this reaction in a normal dietary situation is extremely difficult to predict. For instance, nitrite reacts at acid *p*H with primary, secondary, and tertiary amines, all of which may be present in diet. Dimethylamine is a strong base and does not nitrosate readily at the low concentrations of nitrous acid typical of a normal dietary situation.

For example, Mirvish (*1*) has studied the kinetics of nitrosation and from the maximum amounts of dimethylamine (40 ppm) and nitrite (200 ppm), he has calculated that in a 300-g meal the formation of *N*-nitrosodimethylamine within 3 hours at acid *p*H could be as low as 3 μg—that is, a yield of 0.015 percent based on the amine in the presence of excess nitrite. Such yields are two to three orders of magnitude lower than those usually experienced by the organic chemist. Thus it is necessary to define as precisely as possible the conditions relevant to a dietary situation to determine whether a recognized reaction will take place.

Archer *et al.* (*2*), having examined the reaction of creatine and creatinine with nitrite, conclude that "it remains to be determined whether these reactions," one product of which is the weak rat carcinogen *N*-nitrososarcosine, "actually take place in foods or the mammalian stomach and to evaluate their significance in the incidence of human cancer." It is important that such possibilities are put into perspective in the context of man's contact with the environment.

For instance, the rate of nitrosation of a secondary amine is proportional to the square of the nitrite concentration (*1*), and in the case of anacidity the nitrite concentration in the stomach can reach values of as high as 24 mg per 100 ml of stomach contents (*3*). The nitrite concentration reported for the deliberate nitrosation of creatine to produce *N*-nitrososarcosine was approximately 150,000 ppm. Similarly, the rate of nitrosation of a secondary amine is directly proportional to the concentration of the amine itself (*1*). That of creatine in meat has been reported (*4*) to average 5500 ppm on a wet weight basis, whereas the nitrosation of creatine was conducted at a level of approximately 150,000 ppm. Taking into account the observed effects on nitrosation of both nitrite and creatine concentrations, the rate of conversion of creatine from meat to *N*-nitrososarcosine could be reduced by almost precisely a factor of 10^7 in comparison with the yield of 23 percent during 2½ hours at 25°C reported under favorable conditions. Of the anions known to catalyze the nitrosation of a secondary amine (*5*), thiocyanate is the most important in that it occurs normally in the saliva. If all the thiocyanate of the saliva finds its way into the gastric contents, however, it is unlikely that it will increase the rate of nitrosation by more than one