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.

LARRY L. TRIPLETT
ALAN H. HABER
Biology Division,
Oak Ridge National Laboratory,
Post Office Box Y,
Oak Ridge, Tennessee 37830

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.

24 July 1972

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.

H. MEYER
A. M. MAYER
Department of Botany, Hebrew
University of Jerusalem,
Jerusalem, Israel

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

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

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

96

or possibly at the most two orders of magnitude, which still leaves a large margin even from extreme practical reality.

Although the finding in food or in vivo of even a few parts per billion of a nitrosamine should not be dismissed lightly, it is equally important that the reactions leading to the formation of such compounds be extrapolated on a scientific basis to the actual conditions of the environment in order to evaluate realistically the potential carcinogenic hazard to man.

C. L. WALTERS

British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey, United Kingdom

References

1. S. S. Mirvish, *J. Nat. Cancer Inst.* **44**, 633 (1970).
2. M. C. Archer, S. D. Clark, J. E. Thilly, S. R. Tannenbaum, *Science* **174**, 1341 (1971).
3. J. Sander and G. Buerkle, *Z. Krebsforsch.* **73**, 54 (1969).
4. R. A. Lawrie, *Meat Science* (Pergamon, Oxford, 1966), p. 67.
5. E. Boyland, E. Nice, K. Williams, *Food Cosmet. Toxicol.* **9**, 639 (1971).

Considering the numerous reports of the occurrence of nitrosamines (*1*), we feel that the question of whether nitrosation can occur in the environment hardly has been answered. What should be asked instead is: How much and which N-nitroso compounds occur in the environment? It is to this end that we undertook the research on creatine and creatinine. We carefully avoided ascribing any direct link to human cancer from our results, as quoted in the third paragraph of Walters' comment: "it remains to be determined. . . ."

While we do not essentially disagree with any of Walters' statements, we find it surprising that he derives a sense of either security or insecurity from his oversimplified calculations. We feel the following points merit consideration in determining the value of such calculations.

1) The carcinogenicity of N-nitrososarcosine has been established for only one species of rat (*2*). Essentially all authorities on chemical carcinogenesis would agree that this neither allows the prediction of activity in man, nor the assumption that it is a weak carcinogen, as found for the BD rat.

2) The toxicity of the reaction products of creatinine with nitrite (creatinine-5-oxime and 1-methylhydantoin-5-oxime) have not been tested in any species.

3) Walters takes our yield data and applies correction factors derived from rate considerations to compute an approximate yield of N-nitrososarcosine in the stomach. Since no rate data have been published, the calculations are necessarily invalid. In fact, unpublished rate data from our laboratory indicate that at 37°C and pH 3.5, 20 m*M* creatine (2620 ppm) and 10 m*M* nitrite (460 ppm) yields 20 μM N-nitrososarcosine (2.36 ppm) in 4 hours. A food product, however, may be stored for a relatively long period compared to the time of ingestion and absorption. Given an infinite amount of time and provided that the nature of the reaction and yield of product does not change, creatine at 5500 ppm and sodium nitrite at 240 ppm should give approximately 48 ppm of N-nitrososarcosine (if we assume a 35 percent yield). (Walters' figure of 23 percent for our yield was incorrectly calculated, since nitrite was the limiting reactant, not the creatine.)

4) Although we found a yield of 35 percent of N-nitrososarcosine under our reaction conditions in 2.5 hours, this does not preclude the possibility of having reached a significant fraction of this yield in a much shorter time under the same or more optimum conditions. It also does not preclude the possibility of much higher or lower yields than we reported, particularly since there are a number of parallel and consecutive reactions involved in this system. We have some data indicating that the ratios of reactants are critically important in determining the rate of formation of N-nitrososarcosine and the ultimate yield of product. In addition, since factors such as optimum pH have not been determined, there is at present no means of establishing a priori what the yield would be under the conditions of the stomach or in some food product.

5) Walters assumes that he has considered all of the important factors that can influence rate and yield. He cites thiocyanate as a possible 100-fold accelerating factor. Studies on food or model systems often fail to consider the possibility of unequal distribution of reactants. The sensitivity of nitrosation reactions to hydrogen ion concentration is such that each pH unit from the optimum pH for nitrosation gives approximately one order of magnitude change in reaction rate. These factors and others can lead to such large errors in predicting nitrosamine

concentrations that for most cases it is not worthwhile unless the system is well defined. Since Walters has chosen the environment for his system, this leaves a very large amount of room for error.

6) Walter cites Mirvish's study of dimethylamine nitrosation (*3*). He could hardly have chosen a more negative system. More recent studies on in vivo nitrosation by Mirvish and co-workers (*4, 5*) include the group of compounds: morpholine, piperazine, N-methylaniline, ethylurea, methylurea, and dimethylamine. Each of these compounds was fed to mice along with nitrite in the food or drinking water, and only dimethylamine failed to induce lung adenomas. The basis for this failure is well established in the principles of physical chemistry.

7) Our study on the reaction of creatine and creatinine with nitrite were only intended to establish the nature of the reaction products, and were not even designed to yield information on the probability of their environmental occurrence. We feel that more qualitative studies of this type should be conducted with other types of compounds containing nitrogen to provide a more solid information base for analytical and kinetic investigations in systems of practical importance.

In conclusion, we would agree with Walters' final paragraph. In due time we hope to accumulate sufficient information on kinetics and toxicology to make intelligent predictions and to help us "evaluate realistically the potential carcinogenic hazard to man." We do not believe Walters' calculations are a proper step in this direction.

We wish to call attention to our inadvertent omission of an important article by Greenwood and Levy (*6*) on the reaction of creatinine and some of its derivatives with nitrous acid.

MICHAEL C. ARCHER
STEVEN R. TANNENBAUM

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References

1. I. A. Wolff and A. E. Wasserman, *Science* **177**, 15 (1972).
2. H. Druckrey, R. Preussmann, S. Ivankovic, D. Schmahl, *Z. Krebsforsch.* **69**, 103 (1967).
3. S. S. Mirvish, *J. Nat. Cancer Inst.* **44**, 633 (1970).
4. M. Greenblatt, S. S. Mirvish, B. T. So, *ibid.* **46**, 1029 (1971).
5. S. S. Mirvish, M. Greenblatt, V. R. Choudari Kommineni, *ibid.* **48**, 1311 (1972).
6. I. Greenwood and I. Levy, *J. Org. Chem.* **13**, 554 (1948).