convection is increased. (The efficiency is measured by α, the ratio of the convection mixing length to the pressure scale height. As in most stellar structure calculations, the convection has been modeled by the mixing-length formalism.)

The present discussion is concerned with relatively small changes in the structure of the outer layers of the star, which contain only a small fraction of the total mass. What is required is not the change in period with α for models of the same mass and radius, but rather the change in period for models with the same total mass and same structure in the deep interior. To fulfill the second requirement I have interpolated in the grid of models (*3*), so that the comparison is made between models for which the radius at a chosen mass fraction at the base of the envelope, $M_r/M = 0.55$, is the same.

From the results of stellar evolution calculations, such as those of Becker *et al.* (*4*), one can determine masses and luminosities appropriate to Cepheids of a given period. From a series of models with $M = 6.5\ M_\odot$ (the subscript $\odot$ denotes the sun), luminosity given by $\log L/L_\odot = 3.6$, and $\alpha = 1.0$ or 1.5, which have periods between 6 and 13 days, I have obtained

$$0.006 \lesssim -\frac{d \log P}{d\alpha} \lesssim 0.04$$

The effective temperatures of these models are between 5750 and 5130 K, compared to that of the sun, which is 5700 K. The lower limit of the above derivative applies to the hottest models, where convection is relatively unimportant, so that it may be taken as a minimum value for the influence that changes in convective efficiency will have on the pulsation periods. Upper limits to the dynamic and thermal time scales of the convection zones of these models may also be obtained. The former is less than the pulsation period; the latter is less than 6 years and in most models at least an order of magnitude less.

Photometric observations of many Cepheids over more than 50 years have provided abundant data for the determination of pulsation periods and their change with time (*5*). This has been done by observing the time of maximum light, often to an accuracy of 1 percent of the period, and comparing this with the time predicted by an ephemeris based on a fixed period. From the more than 50 Cepheids that have been investigated in this manner, various patterns of period change emerge. Some stars show no measurable change in period over several thousand cycles. Others exhibit one or more abrupt changes, which occur over a time interval shorter than can be resolved from the data (1 or 2 years). In some cases the change in period appears to happen continuously over 1000 cycles or more.

Period changes resulting from changes in convective efficiency would appear abrupt, since the dynamic time scale is so short. From the published data (*5*), one can obtain an upper limit to the size of abrupt period changes in Cepheids whose periods are in the same range as the models discussed above

$$\Delta \log P \lesssim 1.5 \times 10^{-4}$$

where P is measured in days. From this upper limit and the lower limit of the theoretical derivative, one can infer an upper limit to the changes in α in Cepheid variables

$$\Delta\alpha = \left(\frac{d \log P}{\alpha}\right)^{-1} \Delta \log P < 0.025$$

This may be compared with the value of $\Delta\alpha = 0.02$ which, in Dearborn and Newman's models, produces a change of 1 percent in the luminosity of the sun. Of course, the observed period changes may be due to some other cause entirely, such as the evolution of the star or loss of mass by some ejection process. In that case, the above limit still holds unless changes in convective efficiency occur so rarely as to have never been observed.

Cepheid variables differ from the sun in several important aspects. Although the surface temperatures are similar, the surface gravities of Cepheids are smaller by a factor of 200 or more. (However, Cepheids with higher surface gravities tend to have smaller period changes.) In addition, the hydrodynamic motion of pulsation almost certainly interacts with the convection, and this might affect the possibility of abrupt changes in convective efficiency. Nevertheless, it is of some interest to realize that in these stars any rapid changes of the convective efficiency are most probably smaller than those proposed by Dearborn and Newman for the sun.

BRUCE C. COGAN
Mount Stromlo and Siding Spring Observatories, Woden Post Office, Australian Capital Territory 2606

References and Notes

1. D. S. P. Dearborn and M. J. Newman, *Science* **201**, 150 (1978).
2. R. K. Ulrich, *ibid.* **190**, 619 (1975).
3. B. C. Cogan, *Astrophys. J.* **211**, 890 (1977).
4. S. A. Becker, I. Iben, Jr., R. S. Tuggle, *ibid.* **218**, 633 (1977).
5. P. P. Parenago, *Perem. Zvezdy* **11**, 236 (1956); E. N. Makarenko, *ibid.* **16**, 388 (1968); V. M. Grigorevskij and V. D. Motrich, *ibid.* **19**, 165 (1973).

18 December 1978; revised 2 April 1979

Nitrite Promotes Lymphoma Incidence in Rats

Abstract. *Rats were exposed to sodium nitrite in food or water at concentrations of 0, 250, 500, 1000, and 2000 parts per million. Lymphoma was increased in all groups fed nitrite; the overall combined incidence was 5.4 percent in 573 control rats and 10.2 percent in 1383 treated rats. The mechanism of cancer induction did not appear to be through the formation of nitrosamines but through a more direct effect of nitrite on the lymphocyte.*

A few years ago we found a significant increase in tumors of the lymphoreticular systems of rats fed nitrite (*1*). We felt that this observation (made in the course of an investigation designed primarily to compare the effects of preformed *N*-nitrosomorpholine with those of nitrite plus morpholine) warranted further attention. We designed a study to investigate the effects on the lymphatic systems of rats of nitrite alone and nitrite fed in various concentrations and in different vehicles.

Eighteen groups of rats (Sprague-Dawley CRCD, Charles River Breeding Laboratories) were housed singly in screen-bottom, stainless steel cages with free access to food and water. Groups 1 to 5 received 0, 250, 500, 1000, or 2000 parts per million (ppm) sodium nitrite, respectively, in a semipurified agar gel casein diet, which was described previously (*2*). Groups 6 and 7 were given the same diet, but the nitrite (1000 or 2000 ppm) was administered in the drinking water. Group 8 was a positive control, receiving 2000 ppm urethane in the agar diet. Groups 9 to 11 ate commercial chow (Ralston Purina) containing 0, 1000, or 2000 ppm added sodium nitrite; group 12 was an additional positive control, receiving 2000 ppm urethane in the chow. Groups 13 and 14 were given the same agar gel casein diet as groups 1 to 5, except that it was in a dry form with 0 or 1000 ppm nitrite. Diets in groups 1 to

0036-8075/79/0608-1079$00.50/0

Table 1. Summary of results for rats fed nitrite.

Treatment	Group	Nitrite dose (ppm)	Proportion (and percent) of rats with	
			Malignant lymphomas	Immunoblastic cell proliferation
Semipurified diet (agar gel)	1	0	5/136 (3.7)	10/136 (7.3)
	2	250	10/136 (7.3)	9/136 (6.6)
	3	500	11/136 (8.1)	23/136 (16.9)
	4	1000	11/136 (8.1)	14/136 (10.3)
	5	2000	14/136 (10.3)	23/136 (16.9)
In water (agar diet)	6	1000	16/136 (11.8)	17/136 (12.5)
	7	2000	14/136 (10.3)	18/134 (13.4)
Positive control, 2000 ppm urethane (agar diet)	8		37/135 (27.4)	0/135 (0)
Commercial lab chow (Purina)	9	0	9/132 (6.8)	5/132 (3.8)
	10	1000	14/134 (10.4)	12/134 (8.9)
	11	2000	12/132 (9.0)	11/132 (8.3)
Positive control, 2000 ppm urethane (chow diet)	12		19/136 (14.0)	0/136 (0)
Casein diet	13	0	10/136 (7.4)	10/136 (7.4)
	14	1000	18/136 (13.2)	11/136 (8.0)
Agar diet (mothers of groups 1 and 4)	15	0	1/33 (3.0)	8/33 (24.2)
	16	1000	6/34 (17.6)	8/34 (23.5)
Nitrite exposure after weaning (agar diet)	17	0	6/136 (4.4)	21/136 (15.4)
	18	1000	16/131 (12.2)	18/131 (13.7)

14 were given to the mothers of the experimental and control rats, starting 5 days before they gave birth to their young. When the young were weaned they continued to receive the respective diets until they died or were killed at 6, 12, 18, 24, or (terminally) about 26 months. The rats in groups 15 and 16 were the mothers of the rats in groups 1 and 4 (0 or 1000 ppm nitrite in the agar diet). Groups 17 and 18 were also given 0 or 1000 ppm nitrite in agar but were not exposed to these diets until they were weaned.

Table 1 summarizes this information and gives the tumor incidence in each group. These data are derived from all rats started in the study except for groups 8 to 11, where a few died early in the study and were not considered at risk. All groups except 15 and 16 (mothers) started with 136 animals, equally divided between sexes and randomly distributed from among the mothers within their respective groups. In all, there were 1383 experimental and 573 control rats. Except for animals killed at the predetermined periods of 6, 12, 18, and 24 months, all were allowed to live until they were moribund or dead or were killed when the group was diminished to about 20 percent of the starting number. All slides were viewed and evaluated without knowledge of treatment. Data in Table 1 do not coincide exactly with the figures given in tables 8 and 9 of the final report submitted to the Food and Drug Administration (FDA) (*3*). An additional review of the slides by myself and a colleague, A. Rogers, yielded the numbers shown here in Table 1 which are considered to be more accurate than those in (*3*). A revised report including the data in Table 1 was submitted to the FDA on 25 August 1978.

In this study all malignant tumors of the lymphatic system were combined and categorized as lymphomas. An animal with lymphoma at one or more sites was counted only once in arriving at the incidence. In addition to malignant tumors of the lymphatic system, an alteration referred to as immunoblastic cell proliferation was observed in some members of all groups except the positive controls (groups 8 and 12). This disease in humans (*4*, *5*), which is similar histologically to that observed in our rats, is considered by some to develop into lymphoma (*4*); others consider it nonneoplastic (*5*).

Malignant lymphomas were increased in all groups fed nitrite; the combined incidence of lymphomas in groups that were not fed nitrite was 5.4 percent, compared to 10.2 percent in the combined nitrite-treated groups. The difference in incidence is statistically significant (*6*). Rats with immunoblastic cell proliferation were considered separately, since the significance of this tissue change with respect to developing lymphoma is debatable in humans, and little is known about it in rats.

The observation that nitrite alone increases the incidence of lymphoma is significant since it is apparently the first time that this has been confirmed in a study where numbers of animals and length of study period have been adequate. Furthermore, the pattern of tumors suggests that the carcinogenic effect of nitrite was through a mechanism other than formation of nitrosamines. Nitrosamines characteristically cause cancers to develop at a number of sites, including the esophagus, lung, stomach, liver, urinary bladder, and the central nervous system (*7*). Although a spectrum of tumors characteristic of those reported to be spontaneous in the Sprague-Dawley strain of rat was observed, only the incidence of lymphatic tumors was raised. This pattern has not been reported previously in studies with nitrite, except as an incidental observation (*1*).

The feed samples were analyzed on two different occasions for the presence of nitrosamines by a method with a sensitivity of 10 to 100 parts per billion (*8*); none were detected. Thus, it seems unlikely that preformed nitrosamines were responsible for the observed effect on the lymphatic system. This does not preclude nitrosation in vivo, but this too seemed unlikely since aside from lymphomas, the same spectrum of tumors with similar incidences was observed in both treated and control groups. These data will be presented in detail elsewhere (*9*).

Nitrite is known to cause changes in the size and shape of red blood cells (*10*, *11*). These changes may be related to its effect on hemoglobin, resulting in methemoglobinemia (*12*). Because these alterations in red cells are readily detectable by the immune system (*13*), the animals' immune systems could have become "depressed" in order to maintain red blood cell life. This decreased activity might in turn eventually have led to overcompensation, to the observed proliferation of the immune tissues, and ultimately to tumors. This interpretation of the sequence of events is speculative, but if it is correct, nitrite may not be acting as a "classical" direct carcinogen but rather as an indirect carcinogen or promoter. On the other hand, nitrite, like many know direct carcinogens, is mutagenically active (*14–19*).

Research is needed to confirm that the effects of nitrite observed in the rats in this study are not specific only to this strain of rat or, for that matter, only to rats. Different species or even different strains of the same species can vary significantly in susceptibility to a chemical

challenge. Although there is no evidence that the effects observed in this experiment are species- or strain-dependent, data from a second species would aid in the interpretation of the results of this study and permit more precise estimates of the potential risks to humans from the consumption of nitrite. Such data could be developed only from other feeding studies requiring 3 to 4 years to complete. Since human exposure to nitrite is apparently greater than was previously recognized (*20*), a prudent course of action would include a gradual lessening of nitrite as a food additive as alternative plans are introduced to protect the public from botulism.

PAUL M. NEWBERNE
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. R. Shank and P. M. Newberne, *Food Cosmet. Toxicol.* **14,** 1 (1976).
2. G. N. Wogan and P. M. Newberne, *Cancer Res.* **27,** 2370 (1967).
3. P. M. Newberne, final report on Food and Drug Administration contract FDA 74-181 (May 1978).
4. R. E. Scully, J. J. Galadabine, B. U. McNeely, *N. Engl. J. Med.* **298,** 613 (1978).
5. R. S. Neisman, P. Dervan, C. Haudenschild, R. Jaffe, *Cancer* **41,** 507 (1978).
6. Statistical testing was done by maximum likelihood fitting of all the data (except those for the positive controls) to the single-hit model. Two mathematical models were used. In the first model, probability of tumor development = $1.0 - \exp[\alpha_i - \beta\,(\text{dose})]$, where i indicates the experiment (the nitrite vehicle). For this model the β term was significantly different from zero ($P < .01$) and the overall fit gave a $G^2 = 10.94$ for 9 degrees of freedom ($P = .28$, indicating that the model cannot be rejected). Since the values of the constant α in the first model were similar, a second model was fitted: probability of tumor development = $1.0 - \exp[\alpha - \beta\,(\text{dose})]$. The parameters of this model were estimated to be $\alpha = -2.61\ (\pm 0.13)$ and $\beta = 0.307\ (\pm 0.107)$ (for dose in parts per thousand) and $G^2 = 14.76$ with 14 degrees of freedom ($P = .4$). The procedure followed is described in H. O. Hartley and R. L. Sielken, Jr. [*Biometrics* **33,** 1 (1977)].
7. P. N. Magee, R. Montesano, R. Preussman, *ACS Monogr. 173* (1976), p. 491.
8. A. E. Rogers, J. S. Wishnok, M. C. Archer, *Br. J. Cancer* **31,** 693 (1975).
9. P. M. Newberne, in preparation.
10. J. Musil, *Acta Biol. Med. Ger.* **16,** 388 (1966).
11. H. I. Shuval and N. Gruener, *Am. J. Public Health* **62,** 1045 (1972).
12. H. Druckrey, *Arzeim. Forsch.* **12,** 320 (1963).
13. J. Kersy and B. Spector, in *Immune Deficiency Diseases in Persons at High Risk of Cancer,* T. Fraumeni, Ed. (Academic Press, New York, 1975), p. 55.
14. H. B. Strack, E. B. Freese, E. Freese, *Mutat. Res.* **1,** 10 (1964).
15. S. E. Bresler, V. L. Kalinin, D. A. Perumov, *ibid.* **5,** 329 (1968).
16. W. G. Verly, H. Barbason, J. Dusart, A. Petitpas-Dewandre, *Biochim. Biophys. Acta* **145,** 752 (1967).
17. F. J. deSerres, H. E. Brakman, W. E. Barnett, H. G. Kolmark, *Mutat. Res.* **4,** 415 (1967).
18. S. Abe and M. Sasaki, *Proc. Jpn. Acad.* **53,** 46 (1977).
19. L. Fishbein, *Chemical Mutagens* (Academic Press, New York, 1970), p. 252.
20. S. R. Tannenbaum, D. Fett, V. R. Young, P. D. Land, W. R. Bruce, *Science* **200,** 1487 (1978).
21. Supported in part by FDA contract FDA-74-181. I thank W. Rand for performing the statistical analysis.

29 September 1978; revised 8 January 1979

N-Formyliminodiacetic Acid, a New Compound from the Reaction of Nitrilotriacetic Acid and Chlorine

Abstract. *It has been proposed that nitrilotriacetic acid be substituted for trisodium polyphosphates in detergents as a way to reduce the rate of eutrophication in the Great Lakes Basin. The reaction of nitrilotriacetic acid with chlorine-containing solutions produces a hitherto unknown degradation product,* N*-formyliminodiacetic acid, in high yield. The toxicological and environmental implications of this reaction are unclear.*

In 1972 the Woods Committee, appointed by the U.S. Department of Health, Education, and Welfare to examine available evidence on the possible hazards of nitrilotriacetic acid [NTA or *N*,*N*-bis(carboxymethyl)-glycine] to human health, noted that there were ". . . no reports of mutational studies on possible NTA metabolites or on derivatives that might be formed in the environment prior to human exposure" (*1*, p. 21). One reaction on which information on derivatives was lacking was that of NTA and chlorine-containing solutions (*2*). This reaction might occur in the environment in any of several ways: (i) in wastewater treatment or water purification processes; (ii) in the wash cycle when NTA-containing detergents and commercial bleaches are mixed; or (iii) in the home when these products are mixed in concentrated form by the user or by children, either intentionally or accidentally. Morganthaler and Langguth (*3*) studied the reaction of NTA and chlorine under simulated washing conditions and found that maximums of 10 percent NTA and 20 percent sodium hypochlorite (NaOCl) were lost; the reaction products, however, were not determined. Warren (*4*) studied the kinetics and mechanism of NTA degradation at *p*H 5 to 7 and reactant concentrations comparable to those in the environment. Using derivatization and gas chromatographic-mass spectrometric techniques, he found that the principal products were iminodiacetic acid, glyoxylic acid, and glycine when degradation of the NTA was incomplete.

Our studies of this reaction developed out of our interest in learning whether the same products were formed at *p*H 11 as at *p*H 5. By using high reactant concentrations and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, we were able to examine the solutions in situ, thereby minimizing the risks of destroying more labile intermediates or creating artifacts through manipulation of the solutions.

When 1 g of the trisodium salt of the monohydrate of NTA (Monsanto Industrial, St. Louis) was mixed at room temperature with 15.0 ml of NaOCl solution ([NaOCl] = [NaCl] = 0.7*M*, *p*H 11.0), the solution became exothermic (55° to 60°C) after 30 to 45 seconds, fine bubbles evolved, and the *p*H of the solution dropped to 8.0. The presence of N_2 and CO_2 and the absence of volatile chloramines and NH_3 in the evolved gases were confirmed in separate experiments that allowed porting of the gases directly into a mass spectrometer.

After the bubbling had stopped (< 5 minutes), we examined the solution by ^{13}C NMR spectroscopy. Several new signals (Fig. 1) were observed. Singlets at 171.9, 162.0, and 83.0 parts per million (ppm) (*5*) were identified as formate ($HCOO^-$, a minor component), bicarbonate (HCO_3^-), and formaldehyde (as trioxymethylene), respectively, from spectra of aqueous solutions of these species at *p*H 8.0. The NTA signals were unchanged at 176.8 (carboxyl) and 59.5 (methylene) ppm but had diminished in intensity by 85 percent.

Five signals remained unidentified. The offspin resonance coupling of these signals showed two singlets at 177.1 and 176.1 ppm, a doublet at 166.9 ppm, and two triplets at 53.1 and 48.3 ppm. The relative intensity of these signals remained constant from experiment to ex-

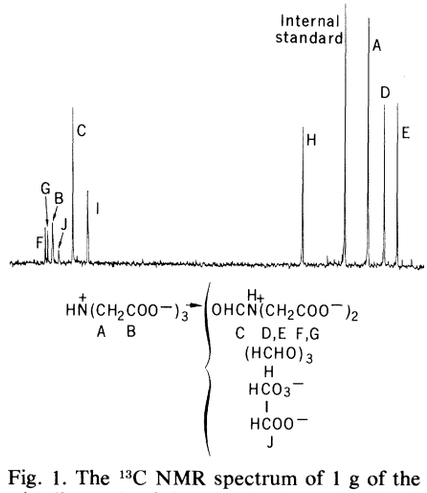

Fig. 1. The ^{13}C NMR spectrum of 1 g of the trisodium salt of the monohydrate of NTA in 15 ml of NaOCl (0.7*M*) solution after 10 minutes of reaction time. The internal standard was dioxane (Varian XL-100 NMR spectrometer).

0036-8075/79/0608-1081$00.50/0