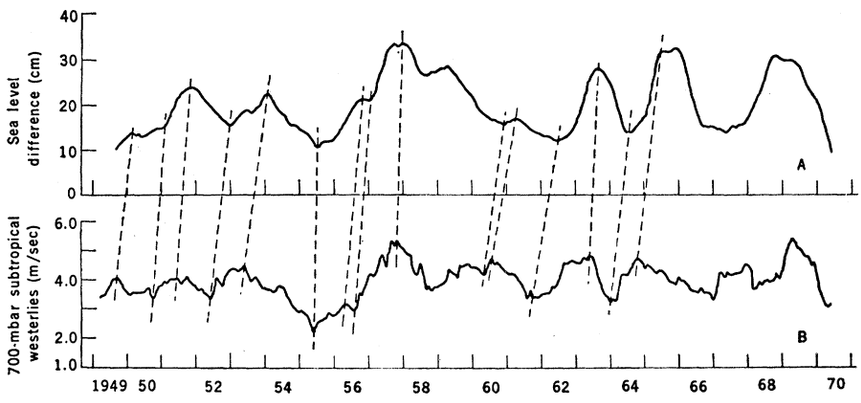

Fig. 1. (A) Annual running means of the sea level difference across the equatorial countercurrent. [After Wyrtki (*1*)] (B) Corresponding values of the strength of the subtropical 700-mbar westerlies as measured between 150°E to 110°W between 20°N 35°N.

correlation with the sea level zonal wind index immediately below is .85 (*2*).

Figure 1A reproduces Wyrtki's graph of the sea level difference across the countercurrent. Figure 1B shows the 700-mbar zonal index (zonal component of the geostrophic mean air flow at 700 mbar between 20°N and 35°N averaged from 150°E to 110°W). To conform to Wyrtki's procedure, the zonal index has been smoothed by taking a running annual mean centered on the indicated month. The correlation coefficient between these two series is .48. Inspection suggests that there is a lag between the two graphs, such that certain singular features in the zonal winds show up later in the countercurrent transport index. The broken lines in Fig. 1 indicate some of these possible connections before 1965, after which time such connections are less prominent or lacking. Lag correlations between the curves yield a maximum of .54 at a 4-month lag with a drop off to .40 at 9 months and .48 at no lag.

Since the strength of the trade winds seems to influence the future strength of the countercurrent and since the countercurrent, in turn, influences the sea surface temperatures off Central America (Wyrtki suggests a 3-month lag for the latter), it is of interest to check the total lag relationship between winds and water temperature. In this case, only the annual means of sea surface temperature centered on January have been examined—this is in accordance with the usual time of emergence of El Niño and the definition of an El Niño year. The best lag correlation, .55, was between these 22 annual means of sea surface temperature and the mean annual subtropical zonal index at 700 mbar centered on May, 8 months earlier. This agrees with the average lag suggested by the broken lines in Fig. 1, about 5 months, plus Wyrtki's 3-month lag. This peak value drops off to .48 at lag 0 and .35 at lag 12.

The significance of these statistics is that the east-to-west wind stress on the equatorial countercurrent, implied by the upper-level westerlies in the subtropics, causes variations in the east-west sea level difference and the countercurrent flow, variations which are reflected in the north-south sea level difference (used by Wyrtki) and at a later time in the surface temperatures in the extreme eastern portion of the countercurrent off Central America. When the subtropical upper westerlies are strong, the trade winds are weak and the countercurrent starts to flow more strongly eastward bringing warm water about 8 months later off Central America. When the subtropical upper westerlies are weak (the trades strong) the countercurrent is slowed down or possibly annihilated. In this case colder water, often associated with upwelling, appears off Central America. These findings, along with Wyrtki's, suggest that when more refined researches are completed the life history of the El Niño may be predictable.

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References and Notes

1. K. Wyrtki, *Science* **180**, 66 (1973).
2. Sea level zonal wind index values are issued as 25-year series by the Extended Forecast Division, National Oceanic and Atmospheric Administration, Rockville, Maryland.
3. Supported by the Office of Naval Research and the National Science Foundation.

18 June 1973

N-Nitrosation by Nitrite Ion in Neutral and Basic Medium

Abstract. *Formaldehyde catalyzed the conversion of various secondary amines to nitrosamines in the* p*H range 6.4 to 11.0. Chloral was also an effective catalyst. The reaction proceeds easily enough to have potential synthetic applications; the proposed mechanism could explain some reported anomalies regarding the synthesis of carcinogenic* N*-nitroso compounds in vivo and in vitro.*

There is considerable debate at present concerning the public health significance of amine-nitrite interactions in the human environment (*1*), but there has been little disagreement regarding the *p*H requirements of such interactions. Investigations on mechanisms have suggested that "the protonation of nitrous acid appears necessary for initiating all nitrosation reactions" (*2, 3*), and it is generally assumed that potentially hazardous quantities of carcinogenic *N*-nitroso compounds cannot be produced unless the interaction of nitrite and amine occurs in acidic medium (*3*).

We describe experiments here that reveal a new dimension to the controversy by showing that nonenzymatic nitrosation occurs smoothly under neutral and basic conditions in the presence of appropriate catalysts. Aqueous buffer solutions of diethylamine, sodium nitrite, and formaldehyde led to significant yields (*4*) of diethylnitrosamine at room temperature over the entire *p*H range studied (*p*H 6.4 to 11.0) (Fig. 1). In the absence of formaldehyde, no nitrosamine could be detected above *p*H 7.5 under these conditions (*5*). The yield is almost independent of hydrogen ion concentration in basic medium, the quantity of product at *p*H 11.0 being 40 percent of that found at *p*H 7.5 (Fig. 1).

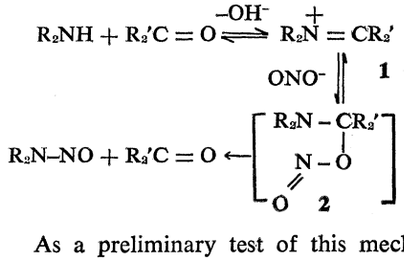
Except for diisopropylamine, all secondary amines we have studied have been easily nitrosated in alkaline formaldehyde solution. Nitrosamine yields varied roughly according to steric accessibility of the nitrogen atom toward

electrophilic attack. We found that the order of decreasing reactivity was pyrrolidine ≃ piperidine ≃ dimethylamine > diethylamine ≃ di-*n*-propylamine >> diisopropylamine.

Under appropriate conditions, the reaction proceeds with enough facility to be useful for preparative applications. When 0.13*M* pyrrolidine was refluxed with 4.0*M* sodium nitrite and 13*M* formaldehyde in 0.1*M* sodium hydroxide for 1 hour, the resulting nitrosopyrrolidine could be isolated in 46 percent yield (*6*). While higher nitrosamine yields are generally possible under milder conditions in acidic media, formaldehyde catalysis could prove crucial in nitrosating amines containing acid-labile functional groups.

Although formaldehyde has been said to "bear little resemblance" to other aldehydes with respect to its reactivity toward amines (*7*), we found that chloral was almost half as effective in catalyzing alkaline *N*-nitrosation, and it is possible that other carbonyl compounds might also perform this function. However, acetone and 2,2-dimethylpropionaldehyde failed to promote observable nitrosation under the same conditions, and we conclude that catalytic effectiveness is strongly dependent on the structure of the carbonyl compound employed.

We propose that the mechanism of catalysis involves initial interaction of aldehyde with the secondary amine (*8*). Among the expected products of this interaction are iminium ions (**1**), which are known to be highly reactive toward nucleophiles (*9*), and which therefore could be expected to undergo attack by nitrite ion. If the adduct, **2**, assumes a conformation in which the two nitrogen atoms can approach each other, direct collapse to the nitrosamine could occur. Tertiary amines and their quaternary salts should not be subject to such catalysis because they are incapable of directly forming aldehyde adducts of structure **1**.



As a preliminary test of this mechanism we prepared *N*,*N*-dimethylformaldiminium (**1**; R = CH_3, R′ = H) trifluoroacetate (*10*) and reacted it with a slight excess of silver nitrite in anhydrous acetonitrile solution. As predicted, dimethylnitrosamine was produced in a yield greater than 90 percent (*11*).

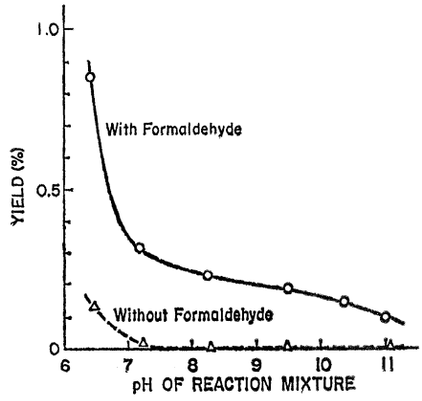


Fig. 1. Percent yield of diethylnitrosamine after 17 hours at 24°C as a function of *p*H in the reaction of 0.05*M* diethylamine with 0.2*M* sodium nitrite (in 0.5*M* phosphate buffer with and without 0.05*M* formaldehyde).

Significant environmental synthesis of *N*-nitroso compounds must now be considered possible even in nonacidic media. Formaldehyde is one of this nation's leading industrial products (*12*) and is widely distributed in our environment (*13*). It is a germicide and fungicide for plants and vegetables (*13, 14*) and is used in combination with nitrite (*15*) as a preservative for fish (*16*). Formaldehyde is formed during the incomplete combustion of many organic substances, and it is especially abundant in smoke used for smoking ham and fish (*14*), two foodstuffs which sometimes contain detectable dimethylnitrosamine (*16*). Chloral is used as a sedative and anesthetic for farm animals (*14*), and a number of other aldehydes which might also catalyze *N*-nitrosation are likely to be found in foodstuffs or other environmental components as well.

Our results hold several additional implications for those studying environmental nitrosation mechanisms. First, adequate descriptions of bacterial or other enzymatic syntheses of nitrosamines must demonstrate, not assume, the absence of catalysis in a boiled system (*17*). Second, since the two curves of Fig. 1 appear to diverge at the low *p*H extreme (that is, at *p*H 6.4, the most acidic solution we investigated), kineticists (*18*) seeking to estimate in vivo nitrosamine yields on the basis of data from in vitro model systems must consider the possibility that this kind of catalysis might also be important in gastric contents and other acidic media. Finally, *N*,*N*-disubstituted iminium ions produced from other than secondary amines should be considered as possible nitrosamine sources by way of their potential reactivity toward nitrite ion (*19, 20*); thus carbon-protonation of the tertiary enamine aminopyrine should give an iminium ion (**1**; R = CH_3, $R_2' = C_{11}H_{12}N_2O$), the reaction of which with nitrite ion could explain the exceptional facility of the drug's conversion to dimethylnitrosamine in vitro (*21*) and in vivo (*22*).

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References and Notes

1. C. L. Walters, *Science* **179**, 96 (1973); M. C. Archer and S. R. Tannenbaum, *ibid.*, p. 97; I. A. Wolff and A. E. Wasserman, *ibid.* **177**, 15 (1972); W. Lijinsky and S. S. Epstein, *Nature* **225**, 21 (1970); S. D. Fine, *Fed. Reg.* **37** (No. 213), 23456 (1972).
2. J. H. Ridd, *Quart. Rev. Chem. Soc.* **15**, 418 (1961); see also (*3*).
3. Nitrosamine formation in neutral medium has been reported to take place in the presence of certain bacteria; compare with G. M. Hawksworth and M. J. Hill, *Brit. J. Cancer* **25**, 520 (1971); J. B. Brooks, W. B. Cherry, L. Thacker, C. C. Alley, *J. Infec. Dis.* **126**, 143 (1972).
4. Nitrosamine production could not have resulted from the generation of hydrogen ion gradients during mixing, because this would have led to initial surges in yield, while we could detect no product in reaction mixtures worked up immediately after thorough mixing.
5. Nitrosamines were extracted with methylene chloride; the absorbancy at the 353 to 358-nm maximum was measured. Yields were corrected for the amount of product known from model experiments to remain unextracted under these conditions. With the use of product isolated from nitrosation mixtures at *p*H 8, the identities of all five nitrosamines produced were confirmed by gas chromatography–mass spectrometry (stainless steel column, 1.8 m by 3.2 mm; packed with 15 percent DEGS on Chromosorb WAW; operation at 120° to 150°C, with helium as carrier gas, at 40 ml/min) on a Hewlett-Packard 5700 gas chromatograph interfaced with a JEOL JMS-01SG-2 mass spectrometer; chromatograms and spectra were in all cases identical with those of reference specimens.
6. A drop of *p*H from 10.8 to 7.2 occurred during the reaction, presumably because of autoxidation of formaldehyde under strongly basic conditions.
7. W. V. Farrar, *Rec. Chem. Progr.* **29**, 84 (1968).
8. L. W. Haynes, in *Enamines: Synthesis, Structure, and Reactions*, A. G. Cook, Ed. (Dekker, New York, 1969), pp. 55–100; M. Masui, K. Fujita, H. Ohmori, *J. Chem. Soc. Chem. Commun.* (1970), p. 182.
9. J. V. Paukstelis, in *Enamines: Synthesis, Structure, and Reactions*, A. G. Cook, Ed. (Dekker, New York, 1969), pp. 169–209; W. P. Jencks, *Progr. Phys. Org. Chem.* **2**, 63 (1964).
10. A. Ahond, A. Cavé, C. Kan-Fan, P. Potier, *Bull. Soc. Chim. France* (1970), p. 2707.
11. Dimethylnitrosamine was identified in the reaction mixture by nuclear magnetic resonance spectrometry and further characterized as described (*5*). The product was quantified by direct measurement of the reaction mixture's absorbance at 351 nm (molar extinction, $e = 112$); the shoulder corresponding to the nitrite ion (320 nm) was nearly undetectable in the spectrum used for quantitation.
12. D. M. Kiefer, *Chem. Eng. News* **50** (No. 51), 9 (1972).
13. L. Fishbein, W. G. Flamm, H. L. Falk, *Chemical Mutagens, Environmental Effects on Biological Systems* (Academic Press, New York, 1970), pp. 206–211.

14. P. G. Stecher, Ed., *The Merck Index* (Merck, Rahway, N.J., ed. 8, 1968), pp. 232 and 468.
15. N. Koppang, P. Slagsvold, M. Aas Hansen, E. Sögnen, R. Svenkerud, *Nord. Vet. Med.* **16**, 343 (1964).
16. Dimethylnitrosamine has been found in some fish products in concentrations high enough to kill domestic animals [N. P. Sen, L. A. Schwinghamer, B. A. Donaldson, W. F. Miles, *J. Agric. Food. Chem.* **20**, 1280 (1972)]. Smaller amounts of this compound have been confirmed in a variety of human foods, including smoked fish [T. Fazio, J. N. Damico, J. W. Howard, R. H. White, J. O. Watts, *ibid.* **19**, 250 (1971)] and smoked ham [T. Fazio, R. H. White, J. W. Howard, *J. Ass. Offic. Anal. Chem.* **54**, 1157 (1971)].
17. Rat cecal contents [P. Klubes and W. R. Jondorf, *Res. Commun. Chem. Pathol. Pharmacol.* **2**, 24 (1971)] and bacterial cultures [D. L. Collins-Thompson, N. P. Sen, B. Aris, L. Schwinghamer, *Can. J. Microbiol.* **18**, 1968 (1972)] nitrosate dimethylamine at neutral or near neutral *p*H even after autoclaving. Catalysis of the type we described might account for at least some of the observed nonenzymatic nitrosamine formation in these cases and could ultimately be important in elucidating the nature of the active site in confirmed cases of enzymic nitrosation.
18. S. S. Mirvish, *J. Nat. Cancer Inst.* **44**, 633 (1970); *ibid.* **46**, 1183 (1971); ——— and C. Chu, *ibid.* **50**, 745 (1973); T-Y. Fan and S. R. Tannenbaum, *J. Agric. Food Chem.* **21**, 237 (1973); M. A. Friedman, *Bull. Environ. Contam. Toxicol.* **8**, 375 (1972).
19. W. Lijinsky, L. Keefer, E. Conrad, R. Van de Bogart [*J. Nat. Cancer Inst.* **49**, 1239 (1972)] have suggested that it may be necessary to revise the accepted (*20*) mechanism of tertiary amine nitrosation.
20. P. A. S. Smith and R. N. Loeppky, *J. Amer. Chem. Soc.* **89**, 1147 (1967).
21. W. Lijinsky, E. Conrad, R. Van de Bogart, *Nature* **239**, 165 (1972).
22. W. Lijinsky and M. Greenblatt, *Nature New Biol.* **236**, 177 (1972).
23. We thank Drs. S. S. Mirvish and W. Lijinsky for bringing two of the references to our attention.

4 May 1973; revised 25 June 1973

Chromatographic Separation of Odorants by the Nose: Retention Times Measured across in vivo Olfactory Mucosa

Abstract. *The column of a standard gas chromatograph was replaced with in vivo frog olfactory sac. The wide range of relative retention times as measured across the olfactory mucosa for 15 different odorants supports the concept of a chromatographic separation along the mucosa as a mechanism for distinguishing different odorants.*

The chromatographic data we report here provides direct evidence that the molecules of different odorants migrate at different rates across the olfactory mucosa. This gives further support to chromatography as one of the models for explaining olfactory discrimination at the level of the olfactory mucosa.

Mozell previously supported this chromatographic model with electrophysiological evidence only. He sampled the activity elicited by different odorants in two widely separated regions of the olfactory mucosa by simultaneously recording the multiunit discharges from the two branches of the olfactory nerve serving those regions (*1*). The more medial branch (MB) reflected the activity at a mucosal region where odorized air first enters the olfactory sac through the external naris. The more lateral branch (LB) reflected the activity farther along the flow path where the air exits from the olfactory sac through the internal naris. The ratio of the discharge magnitude recorded from the lateral branch to that recorded from the medial branch (LB/MB ratio) was used to quantify the gradient of activity across the mucosa which results from an odorant stimulation; the smaller this ratio, the sharper the decline in activity from the entrance region of the mucosa to its exit region. Mozell found that different odorants produced different LB/MB ratios, so that the analysis of odorants might depend in part on the different gradients of activity they establish across the mucosa (*2–4*). They might also be temporally differentiated because the elapsed time (that is, the latency difference) between the onset of the discharges recorded from the two nerve branches also depended on the odorant used. Two further observations influencing Mozell's conclusions were that those odorants yielding the smallest LB/MB ratios also yielded the longest latency differences (*3*) and that reversing the direction of the odorized air flow across the mucosa reversed the nerve branches giving the larger and smaller discharges (*1*).

Mozell suggested that all these observations could be explained by the same underlying mechanism—differences in the rate at which the molecules of different odorants migrate across the mucosa. As Beidler (*5*) suggested earlier in a somewhat different context, perhaps those molecules that are more strongly attracted to the mucosa migrate toward its far end less rapidly (producing longer time lapses) and in fewer numbers (producing smaller LB/MB ratios) than those with less attraction. If the olfactory mucosa can separate the molecules of different odorants by their differing abilities to migrate across it, an analogy could be made between the initial events in olfactory discrimination and those events that are fundamental to chromatography. That is, the analysis of different chemicals by chromatographic techniques likewise depends upon the phenomenon of differential molecular migration which is based upon the differential attraction of molecules to the medium through which they pass.

As one test of this analogy Mozell compared the LB/MB ratios produced by 16 different odorants to the retention times of the same odorants as measured by a standard gas chromatograph fitted with a Carbowax 20M column (*4*). With only one major exception (butanol) those odorants that took longest to migrate through a Carbowax column (that is, those having longest retention times) also had least facility to migrate across the mucosa (that is, produced the smallest LB/MB ratios).

These electrophysiological observations provide only indirect evidence of differential molecular migration patterns across the mucosa since they are made at a level several steps beyond the molecular events that are presumed to initiate them. Furthermore, even if there were, as Moncrieff (*6*) demonstrated in vitro, some chromatographic effect across the mucosa, it is possible that the mucosas of most animals are too short to allow an adequate separation of different odorants. Therefore, we decided to determine whether the molecules of different odorants do indeed migrate at demonstrably different rates across mucosas by measuring directly their relative retention times as they pass along the frog's olfactory mucosa in vivo.

We replaced the standard column of a gas chromatograph (Varian-Aerograph model 600D) with the olfactory sac of an intact frog (*Rana catesbeiana*) anesthetized with urethane. We connected the inlet port of the gas chromatograph to the frog's external naris and the frog's internal naris to the chromatograph's flame ionization detector with Teflon tubing. We also made provision to bypass the frog with this Teflon tubing, thus producing a direct connection from the inlet port to the detector. In either case we determined retention times in the usual manner by measuring the time between the injection of the odorant sample and the maximum pen deflection of the recorded chromatogram, a deflection that signals the arrival of the maximum number of odorant molecules at the