REFERENCES AND NOTES

1. B. A. Smith *et al.*, *Science* **246**, 1422 (1989); H. B. Hammel *et al.*, *ibid.* **245**, 1367 (1989).
2. A. P. Ingersoll, *ibid.* **248**, 308 (1990).
3. J. W. Warwick *et al.*, *Science* **246**, 1498 (1989).
4. S. S. Limaye, L. A. Sromovsky, V. E. Suomi, *Adv. Space Res.*, in press. All narrow-angle frames between 3 and 6 days before encounter (FDS #s 11215.36 to 11305.00) in different filters were used for the measurements of cloud motion. Targets were followed for as long as possible, and linear drift rates were obtained by regression from multiple position and time measurements. Resulting vectors were binned in latitude bands and averaged. The error bars represent ±1 rms deviation within the latitude band.
5. R. C. Tolman and P. C. Fine, *Rev. Mod. Phys.* **20**, 51 (1948).
6. D. R. Johnson, *Adv. Geophysics* **31**, 43 (1989).
7. A. Eliassen, *Astrophys. Norv.*, **5**, 19 (1951).
8. This argument presumes that the time scale of Lagrangian convective transport within the vortex is small relative to the period of vortex rotation.
9. J. L. Tassoul, in *Theory of Rotating Stars* (Princeton University Press, 1978), p. 506.
10. This work was supported by National Aeronautics and Space Administration (NASA)–Jet Propulsion Laboratory contract 957739, NASA grant NGR 189-50-002 from the Planetary Atmosphere Program and NASA grant NAG5-81. We thank F. Bretherton, L. Sromovsky, H. Revercomb, and J. Anderson for discussions as well as an anonymous referee for comments.

4 October 1990; accepted 2 January 1991

Nylon Production: An Unknown Source of Atmospheric Nitrous Oxide

MARK H. THIEMENS AND WILLIAM C. TROGLER

Nitrous oxide in the earth's atmosphere contributes to catalytic stratospheric ozone destruction and is also a greenhouse gas component. A precise budgetary accounting of N_2O sources has remained elusive, and there is an apparent lack of source identification. One source of N_2O is as a by-product in the manufacture of nylon, specifically in the preparation of adipic acid. Characterization of the reaction N_2O stoichiometry and its isotopic composition with a simulated industrial adipic acid synthesis indicates that because of high rates of global adipic acid production, this N_2O may account for ~10 percent of the increase observed for atmospheric N_2O.

NITROUS OXIDE IN THE EARTH'S atmosphere is known to be increasing by about 0.2% per year (*1, 2*), presumably from anthropogenic activity. Although the absolute concentration of nitrous oxide is relatively low [~300 parts per billion by volume (ppbv)], it may influence global climatic processes as a result of one of its stratospheric thermal removal reactions:

$$N_2O + O(^1D) \rightarrow NO + NO \quad (61.7\%) \quad (1)$$

$$\rightarrow N_2 + O_2 \quad (38.3\%) \quad (2)$$

The stratospheric loss rate for reaction 1 is 1.1×10^8 to 1.9×10^8 molecules cm^{-2} s^{-1}, and the total loss rate, including photolysis, is 0.9×10^9 to 1.4×10^9 molecules cm^{-2} s^{-1} (*3*). Nitrous oxide serves as the major stratospheric source of NO, which is unambiguously implicated in catalytic ozone destruction. Second, it is a recognized greenhouse gas that may in the future contribute to an enhanced greenhouse effect by as much as 10% (*4*). Unlike most other trace greenhouse gases and O_3 destructive agents, N_2O does not have an adequately resolved budget. About 30% of the sources have not been identified (*5*). Because of its atmospheric lifetime of approximately 150 years (*6*), identification of all significant sources is particularly important. There are other features of the global N_2O budget that are problematic. The rate of increase of N_2O levels is ~0.9 ppb yr^{-1} in the Northern Hemisphere and 0.7 ppb yr^{-1} in the Southern Hemisphere (*2*). In the Northern Hemisphere, N_2O concentrations are typically 0.8 ppbv higher from April to June than during the rest of the year, possibly because of a large, though undefined, continental anthropogenic source (*2*). A clear resolution of the reason for the hemispheric N_2O difference or seasonality is needed.

With regard to identification of atmospheric trace species, sources, sinks, and transformation mechanisms, stable isotope ratio measurements can provide useful, diagnostic information. For atmospheric N_2O there is, at present, a limited database for the isotope ratios $\delta^{15}N$ (*7–10*) and $\delta^{18}O$ (*10–12*). Nitrogen isotopic measurements reveal that N_2O in maritime air differs from continental air (*8*) and is somewhat variable. This suggests variable source strengths. The processes that determine the ultimate nitrogen and oxygen isotopic composition of N_2O in the ocean have not been identified unambiguously. Denitrification has generally been presumed to be the source of N_2O with high $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios (*9, 11–13*); however, recent simultaneous $\delta^{15}N$ and $\delta^{18}O$ measurements of Pacific oceanic N_2O profiles suggest that the opposite is the case (*10*). The heavy isotopes in oceanic N_2O are depleted, relative to atmospheric N_2O to depths of about 600 m, but significantly enriched in deep and bottom waters. Kim and Craig (*10*) proposed that deep N_2O production by nitrification is simultaneously coupled to a kinetic isotopic fractionation during bacterial respiration, and that this process results in an overall enrichment of heavy isotopes in N_2O. Oceanic regions where denitrification occurs, for example, the eastern equatorial Pacific, are also sites of upwelling and thus can provide a source of N_2O (*14–16*) isotopically similar to tropospheric N_2O (*9*). As concluded by Kim and Craig (*10*), isotopic definition of N_2O in active upwelling sites is needed; in consideration of present uncertainties in the atmospheric N_2O budget (*5*), identification of all significant N_2O sources with concomitant isotopic measurements is needed.

On first consideration one might conclude that industrial sources of N_2O are insignificant because of its minor usage (*17*). Its chief application, as a nontoxic propellant in canned whipping cream, does not constitute a significant atmospheric pollution source. In this report, we call attention to an industrial source of N_2O that has atmospheric significance. Nitrous oxide is a by-product in the manufacture of monomers for 6,6- and 6,12-nylon. In 1989 1.24×10^9 kg of nylon were produced in the United States alone (*18*). Nylon polymers have typically been formed by condensation polymerization of a dicarboxylic acid and diamine. The most widely used diacid, adipic acid, is prepared primarily by air oxidation of cyclohexane to cyclohexanol-cyclohexanone mixtures, followed by oxidation with N_2O to adipic acid (*19*). In 1989, U.S. production of adipic acid totaled 7.44×10^8 kg (*18*). Because western European and Japanese production essentially equals U.S. production (*19*), we might assume that other world sources, such as China, the Soviet Union, and Eastern Europe have a total adipic acid output comparable to the United States This suggests a 1989 worldwide production of 2.2×10^9 kg of adipic acid, which agrees with an estimate of 2.2×10^6 tons, or 2.0×10^9 kg worldwide for 1983 (*20*). We have focused on the nitric acid oxidation step involved in adipic acid synthesis because it results in the stoichiometric production of N_2O on a large scale.

Most earlier workers have reported indeterminate stoichiometries for the N_2O produced by the nitric acid oxidation of cyclohexanol-cyclohexanone mixtures, as shown in Eq. 3.

Department of Chemistry, University of California, San Diego, La Jolla, CA 92093.

$$C_6H_{11}OH/C_6H_{10}O + HNO_3 \rightarrow \quad (3)$$
$$HOOC(CH_2)_4COOH + aN_2 + bNO + cNO_2 + dN_2O$$

Some CO_2 is also produced as a by-product from decomposition. Ideal stoichiometries of 0.5 and 1.0 mol of N_2O per mole of adipic acid product have been suggested for the oxidation reaction (*19, 21*). Although the toxic NO and NO_2 gases produced have been targeted for containment in U.S. facilities, the inert N_2 and N_2O are released in unknown quantities. We have been unable to find values for N_2O emissions because they are regarded as harmless by regulatory agencies. Because of the sheer volume of the industrial process, the long residence time of the gas in the atmosphere, and its accompanying consequences, the assumption that such emissions are harmless may be invalid. In countries with relaxed controls on gaseous emissions, the N_2O released could be near the stoichiometric amount.

The uncertainty in the stoichiometry of Eq. 3 and the unknown isotopic composition of the N_2O produced in this process prompted us to define better these unknowns. It was impossible for us to reproduce exactly the constant feed industrial process at an elevated pressure and quantify the gas amounts released. As a rough approximation, we prepared a 50% by weight nitric acid solution that contained 0.01 g of NH_4VO_3 and 0.01 g of Cu metal dissolved in each 5.2 ml of solution. The NH_4VO_3 and Cu metal serve as oxidation catalysts in a common version of the industrial process (*22*). After we degassed 1.3 ml of this solution three times with a freeze-pump-thaw cycle, 0.25 to 0.35 g of cyclohexanol or cyclohexanone was added onto the acid-catalyst solution in a 1-l flask (with a vacuum stopcock) precooled with liquid N_2. The flask was evacuated and then placed in a water bath behind a safety shield at 80 to 90°C for 0.5 hour (a typical residence time for 90% conversion). Although it is not normally advisable to perform the reaction between layers of both substances in a closed system because of the explosion hazard, it did permit us to collect and quantify the N_2O evolved and determine the stoichiometry involved in Eq. 3. After reaction, the flask was connected to a vacuum line, which contained a Toepler pump. About 2.5 mol of gas were evolved per mole of cyclohexanol. Of this, 12% were noncondensible gases (N_2 and CO) at 77 K. From the color of the remaining condensable gas it was apparent that there was little brown NO_2 present; however, when oxygen was admitted to the gas mixture, an intense brown color characteristic of NO_2 appeared. The NO_2 could be separated from the remaining N_2O and CO_2 by collecting the N_2O-CO_2 mixture through two U-traps cooled to −90° to −95°C. The separation step was necessary because NO and NO_2 can react with mercury in the Toepler pump. Oxidation of NO to NO_2 was necessary because NO cannot be cryogenically separated from N_2O, which has a similar vapor pressure. About 30% of the total gas evolved was estimated to be NO. The identity of the N_2O and CO_2 collected was conclusively established from their characteristic gas-phase infrared spectra.

We measured the isotopic composition of N_2O as follows. Nitrous oxide was cryogenically transferred to a nickel reaction tube (~80 cm^3) on a BrF_5 reaction manifold, essentially identical to that developed for silicate oxygen isotopic analysis (*23*). Heating at 700° to 750°C overnight, quantitatively converted N_2O to NiO and N_2. The N_2 was removed at room temperature, and the yield was manometrically measured. Carbon dioxide, which is introduced initially with the N_2O, was not decomposed, and was removed with the N_2. The N_2/CO_2 ratio was determined mass spectrometrically and the stoichiometry of CO_2 production thus obtained. The residual NiO was subsequently reacted overnight with BrF_5 (tenfold excess) at 500°C, which quantitatively converts NiO to O_2 and NiF_2. After O_2 purification, as in (*24*), the yield was determined and $\delta^{18}O$ was subsequently determined, by mass spectrometry. Complete conversion of N_2O to O_2 was observed for all reported data. The measurements were done on a Finnigan MAT 251 mass spectrometer. In the oxidation of cyclohexanol, the products CO_2 and N_2O were 26% and 30%, respectively, of the initial evolved gas. Thus, per mole of organic substrate we obtained 0.65 mol of CO_2 and 0.75 mol of N_2O. Similar experiments with cyclohexanone as substrate at 80° to 90°C showed that 0.30 mol of CO_2 and 0.70 mol of N_2O were produced per mole of substrate. The large amount of CO_2 obtained in these experiments suggests that some additional decomposition of product occurred. When cyclohexanol was oxidized at 65°C for 20 min the yield of CO_2 was reduced to 0.10 mol per mole of substrate and the N_2O yield was 0.50 mol per mole of substrate. On the basis of the adipic acid produced (isolated in 46% yield), the N_2O stoichiometry is 1.09 mol per mole of product. Similar experiments at 65°C with cyclohexanone showed that 0.12 mol of CO_2 and 0.95 mol of N_2O were produced per mole of adipic acid (isolated in 71% yield). The oxygen isotopic composition of the product N_2O ranges between 16 to 20 per mil (SMOW), for the 80°C reaction with both cyclohexanol and cyclohexanone, which is quite distinct from typical tropospheric N_2O values of ~44 per mil (*10–12*). For the 65°C cyclohexanol reaction the $\delta^{18}O$ of the N_2O is 26 per mil. The isotopic values obtained from the experiment are probably representative of those for industrial N_2O; however, there is the possibility that differences in nitric acid compositions may produce different isotopic compositions.

On the basis of experiments, as well as the most recent estimate (*20*), the reaction stoichiometry for N_2O production in the preparation of adipic acid is about 1 mol of N_2O per mole of product. For a global yearly adipic acid production of 2.2×10^9 kg yr^{-1}, this corresponds to about 1.5×10^{10} mol of N_2O by-product. If this value is compared to the annual atmospheric N_2O increase (*1, 6*) of 14.6×10^{10} mol yr^{-1}, this N_2O production would then account for 10% of the observed annual increase of tropospheric N_2O. In that N_2O emissions are not regulated (*25*), it is not known at present what proportion of the N_2O by-product is removed before atmospheric venting, thus our estimate represents an upper limit. A few companies (for example, Monsanto) treat the emissions in a reductive furnace, which destroys N_2O. Thus, the technology is available to solve the problem. Even if, for example, all U.S. adipic acid production plants effectively removed N_2O, there remain significant worldwide sources. Commercial nylon production began in 1939 (*20*); thus, with a ~150-year atmospheric lifetime for N_2O, (*6*) a significant fraction of all N_2O globally associated with nylon production still remains in the atmosphere. There is about a 0.2 ppb yr^{-1} unexplained difference, or $\sim 3.66 \times 10^{10}$ mol yr^{-1}, in the increase in N_2O concentrations between the Northern and Southern hemispheres, (*2, 26*). If one attributes most adipic acid production to the Northern Hemisphere, this could account for 42% of the difference. In that the $\delta^{18}O$ (16 to 26 per mil) of N_2O produced by either the cyclohexanol or cyclohexanone reactions is quite distinct from overall the $\delta^{18}O$ of N_2O (~44 per mil) in the Northern Hemisphere, it would be interesting to compare both hemispheric $\delta^{18}O$ and $\delta^{15}N$ values for N_2O along with variations in rural and urban locations, particularly in locales concerned with nylon production. As concluded by Cicerone (*6*), isotopic measurements of N_2O are needed to address the outstanding questions regarding sources. Although N_2O production from adipic acid synthesis does not completely account for the budgetary mismatch, it does bring attention to a previously unrecognized and potentially significant source. As more sources become identified,

along with their isotopic signatures, a more adequate budget may be constructed. The present measurements establish both a source magnitude and isotopic composition. In consideration of the participation of N_2O as both a catalytic ozone destructive agent and a greenhouse gas, definition of all sources, particularly those which may now be unrestricted, is clearly important. Our analysis suggests that in the long term it may be desirable to develop catalytic oxidation methods that avoid the stoichiometric use of nitric acid as an oxidant.

REFERENCES AND NOTES

1. R. F. Weiss, *J. Geophys. Res.* **86**, 7185 (1981).
2. M. A. K. Khalil and R. A. Rasmussen, *Tellus* **35B**, 161 (1983).
3. P. J. Crutzen and U. Schmailzl, *Planet. Space Sci.* **31**, 1009 (1983).
4. R. E. Dickinson and R. J. Cicerone, *Nature* **319**, 109 (1986).
5. M. B. McElroy and S. P. Wofsy, in *Tropical Rain Forests and World Atmosphere*, G. T. Prance, Ed. (American Association for the Advancement of Science, Washington, DC, 1986), p. 33.
6. R. J. Cicerone, *J. Geophys. Res.* **94**, 18,265 (1989).
7. The delta notation is defined as: $\delta^{18}O = (R^{18}/R^{18}_{STD} - 1) \cdot 1000$; $\delta^{15}N = (R^{15}/R^{15}_{STD} - 1) \cdot 1000$, with $R^{18} = {}^{18}O/{}^{16}O$ and $R^{15} = {}^{15}N/{}^{14}N$. The subscript "STD" refers to the conventional standard, which for oxygen is standard mean ocean water (SMOW) and for nitrogen, is air N_2.
8. N. Yoshida and S. Matsuo, *Geochem. J.* **17**, 231 (1983).
9. N. Yoshida *et al.*, *Nature* **307**, 442 (1984).
10. K.-R. Kim and H. Craig, *Nature* **347**, 58 (1990).
11. M. Wahlen and T. Yoshinari, *ibid.* **313**, 780 (1985).
12. T. Yoshinari and M. Wahlen, *ibid.* **317**, 349 (1985).
13. N. Yoshida, *ibid.* **335**, 528 (1988).
14. J. W. Elkins *et al.*, *ibid.* **275**, 602 (1978).
15. R. F. Weiss, *Eos* **62**, 894 (1981).
16. J. D. Cline, J. D. Wisegarver, K. Kelly-Hansen, *Deep-Sea Res.* **34**, 857 (1987).
17. D. F. Shriver, P. W. Atkins, C. H. Langford, *Inorganic Chemistry* (Freeman, New York, 1990), p. 382; R. R. Beard, in *Patty's Industrial Hygiene and Toxicology*, G. D. Clayton and F. E. Clayton, Eds. (Wiley-Interscience, New York, 1982), pp. 4096–4097.
18. *Chem. Eng. News* **68**, 4 (1990).
19. H. Ulrich, *Raw Materials for Industrial Polymers* (Hansen, New York, 1988), pp. 138–141; J. K. Stille and T. W. Campbell, *Condensation Monomers* (Wiley-Interscience, New York, 1972), pp. 36–53.
20. D. D. Davis, in *Uhlman's Encyclopedia of Industrial Chemistry*, W. Gerhartz, Ed. (VCH, New York, 1985), vol. 1A, pp. 269–278; V. D. Luedeke, in *Encyclopedia of Chemical Process and Design*, J. J. McKetta, Ed. (Dekker, New York, 1977), vol. 2, pp. 128–146.
21. I. V. Berezin *et al.*, *The Oxidation of Cyclohexane* (Pergamon, Oxford, 1966), pp. 251–255.
22. G. W. Parshall, *Homogeneous Catalysis* (Wiley, New York, 1980), pp. 193–195.
23. R. N. Clayton and T. K. Mayeda, *Geochim. Cosmochim. Acta* **27**, 43 (1963).
24. S. K. Bhattacharya and M. H. Thiemens, *Z. Naturforsch.* **44a**, 435 (1989).
25. *Protection of Environment*, Code of Federal Regulations, vol. 40, parts 300–399 (U.S. Government Printing Office, Washington, DC, 1989), pp. 265–273; *Toxic Release Inventory, CD-ROM* (U.S. Environmental Protection Agency, Washington, DC, 1989).
26. *Global Ozone Research and Monitoring Project*, No. 16, VI (World Meteorological Organization, Washington, DC, 1985), vol. 1, p. 78.

27. We thank J. K. Bashkin and R. Cicerone for helpful discussions. Q. W. Dong is acknowledged for laboratory assistance. We also thank two anonymous reviewers. This work was supported by the National Science Foundation (grant CHE-88-15958 to W.C.T. and ATM 87-21051 to M.H.T.).

14 August 1990; accepted 12 December 1990

Distinctive Cranial and Cervical Innervation of Wing Muscles: New Evidence for Bat Monophyly

J. G. M. THEWISSEN AND S. K. BABCOCK

The traditional view that Old World fruit bats (Megachiroptera) and insect bats (Microchiroptera) are closely related has been challenged by claims that Megachiroptera are the sister group to flying lemurs (Dermoptera) or Primates. We found that the specialized muscles of the rostral part of the wing in Microchiroptera and Megachiroptera receive double innervation by both the facial nerve and cervical spinal nerves, suggesting that bats are monophyletic. Innervation by the facial nerve also occurs in Dermoptera and suggests that bats and Dermoptera share a common ancestor that had wings.

BATS (CHIROPTERA) ARE THE ONLY mammals that fly actively, and this locomotor behavior has exerted a pervasive influence on their evolution. A striking aspect of wing morphology is the presence of a specialized muscle complex, sometimes called occipito-pollicalis in bats, that extends along the rostral part of the wing (propatagium) and is absent in quadrupedal mammals (*1*) (Fig. 1). This propatagial muscle complex has been used as evidence for the monophyly of bats (*2*) and to demonstrate close phylogenetic ties between *Cynocephalus* (Dermoptera) and bats (*3, 4*). But the complex is also present in other winged vertebrates that are not closely related, such as the flying squirrel *Glaucomys* (*5*) and many birds (*6*). This suggests that the complex evolved independently in unrelated flying and gliding vertebrates and, thus, may devalue its presence as a supporting character for the monophyly of Micro- and Megachiroptera. Elimination of characters related to aerial locomotion has been used to support claims based on independent evidence that Megachiroptera are more closely related to Primates than to Microchiroptera (*7, 8*).

We used innervation as a criterion for establishing muscle homology because of the close link between innervation and muscle development. Resulting hypotheses of homology form a test for existing cladograms of the phylogenetic relations among archontan mammals (Recent Megachiroptera, Microchiroptera, Dermoptera, Scandentia, and Primates). We studied the innervation of the elements of the propatagial muscle complex using gross dissection and histological serial sections because previous reports are few and inconsistent (*3, 9*). We determined the innervation in the dermopteran *Cynocephalus volans*, the megachiropteran *Pteropus sp.*, and the microchiropterans *Myotis lucifugus* (Vespertilionidae) and *Tadarida brasiliensis* (Molossidae).

The results of our investigation are summarized in Fig. 2. The propatagial muscle complex of *Cynocephalus* consists of two layers extending perpendicular to each other and innervated by different nerves: the dorsal layer by cranial nerve VII and the ventral belly by one or more cervical spinal nerves. In *Pteropus*, five muscle bellies make up the propatagial complex. Four of the bellies are innervated by both cranial nerve VII and cervical spinal nerves. The remaining pectoral belly is innervated by cranial nerve VII and the pectoral nerve. Only two muscle bellies are present in *Myotis*; both are innervated by cranial nerve VII and cervical spinal nerves. The occipital, brachial, and distal bellies of the propatagial muscle complex of *Tadarida* are innervated by cranial nerve VII only, and its pectoral belly is innervated by the pectoral nerve only.

The innervation of the propatagial muscle complex is unusual for two reasons. It is the only known case in which voluntary limb muscles are innervated by cranial nerve VII. In addition, the dual innervation of the propatagial complex in *Myotis* and *Pteropus* by both cranial nerve VII and cervical spinal nerves is unique among mammals. The cell bodies of cranial nerve VII generally reside in the pons and medulla oblongata (*10*) and are not continuous with those of the spinal nerves. Innervation by a combination of nerves from the brain and spinal column has not been reported for any other mammalian muscle. A superficially similar case of dual

Department of Biological Anthropology and Anatomy, Duke University Medical Center, Durham, NC 27710.