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6. Bioluminescent countershading never has been observed from submersibles in spite of numerous observations of midwater animals [for example, R. H. Backus, J. E. Craddock, R. L. Haedrich, D. L. Shores, J. M. Teal, A. S. Wing, G. W. Mead, W. D. Clarke, *Science* **160**, 991 (1968); E. G. Barham, in *Proceedings of an International Symposium on Biological Sound Scattering in the Ocean*, G. B. Farquhar, Ed. (Government Printing Office, Washington, D.C., 1971), pp. 100–118; R. Church, *Oceans* **3**, 20 (1970)]. The failure to observe bioluminescent countershading in nature could be considered as evidence against the theory. We found that countershading was extremely difficult to observe in the aquarium with the observers' dark-adapted eyes only 30 to 45 cm from the animals. With faint overhead illumination in the laboratory, squid could be confirmed to luminesce only when seen against the dimmer portions of the illumination. Such uneven illumination certainly is unlikely to occur in the midwaters of the open ocean. Countershading, such as we observed, is so effective that under natural conditions the animals would be invisible to the observer from all angles. Thus, one should not expect casual observations from a submersible to reveal countershading luminescence. For the present, at least, bioluminescent countershading is best studied in the laboratory.
7. J. W. Hastings, *Science* **173**, 1016 (1971).
8. The ventral surfaces of the mantle, funnel, head, and the ventral two pairs of arms bear numerous, small ventrally directed photophores of three basic types. One type, numbering about one-half of the total photophores, possesses a distal color filter indicative of the countershading function. (The structure of these photophores is currently being investigated by R. E. Young, C. F. E. Roper, and J. Arnold.) In addition, five flattened photophores occur on each eyeball, and a series of three large, spherical photophores occurs on the tip of each ventral arm.
9. R. E. Young, in preparation.
10. ———, *Symp. Zool. Soc. London*, in press.
11. Observations were made separately by each investigator on a total of six squid during a 10-day cruise off Oahu, Hawaii, in November 1975.
12. Time periods with the light on varied from 2 to 16 minutes (mean, 9.7 minutes; 73 percent of trials were between 9 and 11 minutes). Periods with the light off ranged from 5 to 25 minutes (mean, 10.1 minutes; 76 percent of trials were between 9 and 11 minutes). Specimens were placed in the observation tank with the overhead light off at least ½ hour before testing; the observer dark adapted during this period. In all six cases, the squid at this stage did not luminesce.
13. Bioluminescent countershading may be a continuous process, or it may be an intermittent display as part of an escape reaction involving retreat and then concealment (*4*). The consistent reaction of squid in this study to the overhead illumination was unrelated to any apparent escape reaction, supporting the likelihood of a continuous and automatic countershading behavior in these animals. The squid *Heteroteuthis hawaiiensis* under constant overhead illumination in the aquarium (i) produce a luminous cloud, (ii) dart quickly across the tank, (iii) produce a ventral glow for several minutes, and finally (iv) extinguish the glow. This four-step sequence was observed about 20 times in two specimens when apparently they were disturbed repeatedly. In spite of difficulties introduced by the experiment, these observations suggest that intermittent countershading (as part of an escape reaction) remains a distinct possibility for some species.
14. We thank the officers and crew of the R.V. *Kana Keoki*, University of Hawaii, and the members of the scientific party who participated in cruise *Fido* IV for their assistance and Alan Hart, who prepared the illustration. This work was supported in part by National Science Foundation grant DES 72-01456 AO2 (R.E.Y.) and by the Smithsonian Institution (C.F.E.R.). This is Hawaii Institute of Geophysics contribution No. 738.

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# Heavy Methanes as Atmospheric Tracers

Abstract. *Methane-21 ($^{13}CD_4$) is potentially a useful nonradioactive tracer for testing atmospheric transport and diffusion models on a continental scale. In an experiment to demonstrate this long-range utility, the release of 84 grams of methane-21 was detected at distances of 1500 to about 2500 kilometers at concentrations of about 1 part in* $2 \times 10^{16}$ *parts (by volume) in the air by a technique in which methane was separated and the methane-21 content was measured with a mass spectrometer.*

There is a growing need to understand the details of atmospheric circulation as well as the details of the dispersion of pollutants on regional, continental, and global scales. Although much theoretical work has been done on these problems, there have been almost no successful controlled experiments on these scales partly because of the lack of suitable tracer materials. Tracers that have been used successfully at shorter distances are generally impractical for long-range applications because of cost, relatively high background concentrations, interference from industrial sources, or insufficient detection sensitivity. An ideal tracer would be nontoxic and nondepositing, would have virtually no existing background concentration in the atmosphere, would be detectable at extremely low concentrations, and would be economical in terms of the costs of tracer and sample analysis.

With the availability of relatively cheap separated isotopes of carbon (*1*), methane-21 ($^{13}CD_4$) would appear to meet many of these requirements for an ideal meteorological tracer. Natural methane, mostly methane-16 ($^{12}CH_4$), is present in the troposphere (northern mid-latitudes) at a concentration of about 1.4 parts per million (ppm) (*2*), although significant variations in the abundance occur between urban and rural areas (*3*). The production rate of natural methane is estimated to be (*2*) about (0.5 to 1.0) × $10^{15}$ g per year, and is balanced by destruction (*2*) in the troposphere and stratosphere, leading to an estimated mean residence time for methane in the troposphere of 4 to 7 years. The exchange of carbon or hydrogen atoms between the different isotopic forms of methane should be much slower than the apparent destruction rate (*4*). The statistical equilibrium abundance ratio of natural methane-21 to natural methane-16 has been calculated to be $7 \times 10^{-18}$ (*5*). This figure implies that, before the large-scale production of deuterium starting in the 1940's, the world's atmosphere contained only about 50 mg of methane-21. Nuclear energy activities have presumably perturbed this number significantly.

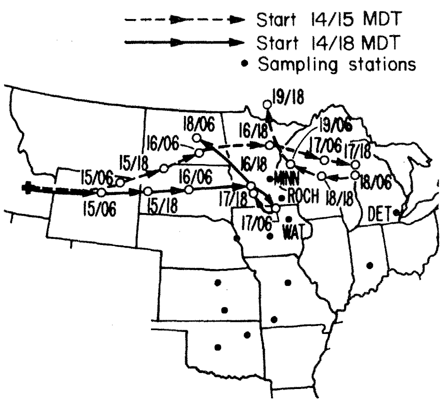

Fig. 1. Post-facto meteorological trajectories, 300 to 2000 m above the ground, starting time 14 May 1974, 1500 and 1800 M.D.T., designated in the figure as 14/15 and 14/18, respectively [see (*7*)], and methane sampling stations. The fractional symbols give the times of arrival of a trajectory at a location; for example, 15/06 means arrival on 15 May, 0600 local time.

The potentialities of methane-21 as a meteorological tracer derive from this low abundance of methane in the atmosphere and from the high sensitivity of an ultrahigh-vacuum, high-resolution mass spectrometer (*6*) fitted with an electrostatic retardation lens. In this instrument, the background in the methane-21 mass region is about $10^{-11}$ of the signal in the methane-16 mass region.

In order to demonstrate the feasibility of large-scale meteorological tracing based upon the use of methane-21, 84 g were released on 14 May 1974, from 1520 to 1750 M.D.T. from a 70-m stack at the Allied Chemical Corporation processing plant at Idaho Falls, Idaho. The initial dilution with air in the stack produced a starting ratio of methane-21 to methane-16 of ≈ 0.1.

Cryogenic air samplers, operating at 13 National Weather Service stations at locations extending from Minnesota to Oklahoma (see Fig. 1), were used to concentrate the methane and krypton in the ambient air. Samples were collected twice daily at each station, from 9 p.m. to 7 a.m. (P) and 9 a.m. to 7 p.m. (A). On the basis of meteorological predictions, about 25 samples from four locations were chosen as likely to contain fractions of the released tracer. Eight additional samples were chosen for background measurements, with the sampling done either before the release or at a place (Tulsa, Oklahoma) far from the predicted trajectories.

Methane and krypton were separated from the air samples and purified by chromatographic desorption, and the isotopic composition of the methane was measured by mass spectrometry. The ratios of the intensities at the mass-21 region characteristic of methane-21 to those at mass 16 for the different samples are presented in Fig. 2. The data shown have been corrected for spectrometer detector noise. The standard deviations $\sigma$ indicated in Fig. 2 are estimates based on the counting statistics and the performance of the instrument during the measurements. Because of an equipment problem at Rochester, Minnesota, the 15 May P and 16 May A samples were not obtained.

Figure 2, b and e, shows that the eight samples that came from air sampled at times or at a place not subject to tracer influence had methane-21/methane-16 ratios of less than $3 \times 10^{-11}$ [average = $(0.7 \pm 1.2) \times 10^{-11}$]. All the other samples, selected as likely to contain the tracer, show higher values of this ratio, with more than half of them yielding ratios in excess of $4 \times 10^{-11}$. The average value observed in these samples, $4.5 \times 10^{-11}$, corresponds to the detection of about 1 part in $2 \times 10^{16}$ parts (by volume) of methane-21 in the atmosphere. Although the abundance of methane in the air corresponding to these samples was not determined directly, the ratio of recovered methane to recovered krypton was the same (1.04) in all the samples to within 12 percent (1 $\sigma$), with no significant correlation of methane-21 contents with recovered methane/krypton ratios. This result makes unlikely any effect of variable methane content (*3*) in the atmosphere on the present conclusions.

Although this methane-21 test was not designed primarily as a meteorological experiment, it is interesting to compare the measured concentrations with those calculated on the basis of a simple model (*7*). The continuous methane release of the experiment was simulated by two puffs, each consisting of 42 g of methane-21, transported along the calculated trajectories shown in Fig. 1. Based on the assumptions that the horizontal standard deviation $\sigma_H$ of the concentration distribution in the puffs grows linearly with time ($\sigma_H = 1.9 \times 10^3\ t$, where $\sigma_H$ is in meters and $t$ is the transit time in hours) and that the puffs are uniformly mixed through a height of 2 km, concentrations were calculated for each sampling period at each station. In the calculations, the methane concentration in the sampled air was assumed to be 1.4 ppm, although the fact that certain of the sampling sites were rather close to airports or urban areas might suggest that a higher value would have been more appropriate (*3*). The ratios of methane-21 to methane-16 calculated in this way are also presented in Fig. 2. In view of the uncertainties in both the calculations and the experimental data, there is fairly good agreement between them. At three stations (Minneapolis, Minnesota; Rochester, Minnesota; and Waterloo, Iowa) the sum of the recovered methane-21 (corrected for background ratios of $0.7 \times 10^{-11}$) in all the samples analyzed is about half of the value calculated. At these three stations the peaks in the observed concentrations occur within 12 hours of the times calculated. For Detroit, Michigan, more methane-21 was found experimentally than calculated, suggesting that the air trajectories from the release site passed closer to Detroit than indicated in Fig. 1. In all cases the observed variability is smaller than that calculated with the simple meteorological model.

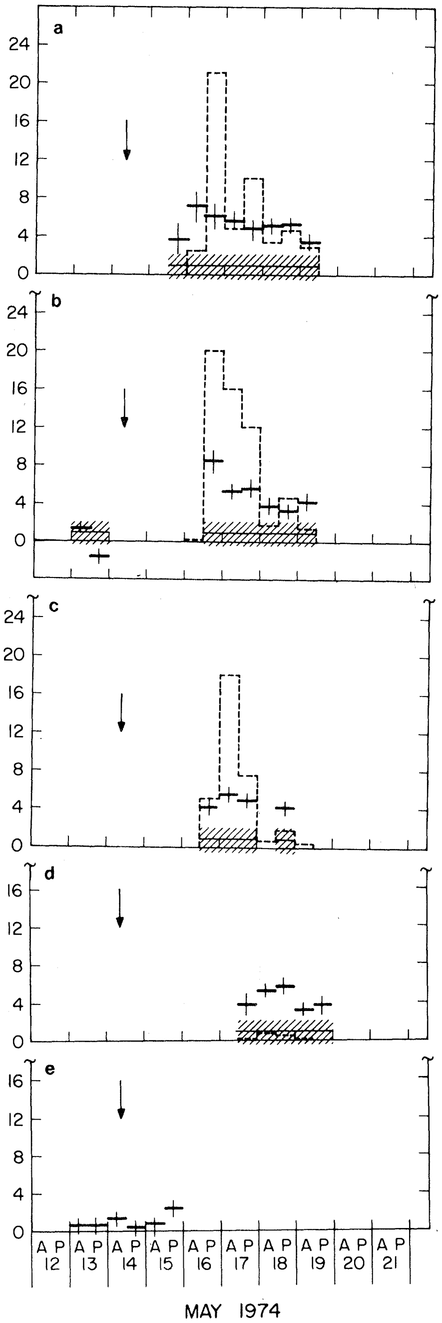

Fig. 2. The observed ratios of methane-21 to methane-16 ($\times 10^{11}$) in air taken at various times at the following locations (heavy bars with standard errors indicated): (a) Minneapolis, Minnesota; (b) Rochester, Minnesota; (c) Waterloo, Iowa; (d) Detroit, Michigan; and (e) Tulsa, Oklahoma. Calculated values based on the model described in the text are represented by the dashed bar graphs. The calculated values for Detroit are all $< 1 \times 10^{-11}$. The time of release of the tracer in Idaho is indicated by the arrows. The horizontal lines with shading indicate the background levels (with standard deviations) observed in samples not affected by the tracer.

In spite of discrepancies between the details of the observations and calculations, Fig. 2 clearly shows that methane-21 has been detected at distances of 1500 to 2500 km from the point of release. This experiment thus demonstrates that isotopically labeled methane can be used on a continental scale to study atmospheric transport and diffusion. Such labeled hydrocarbons have important characteristics to recommend them for these studies. (i) The detection limits are comparable to those theoretically achievable with the use of radioactive tracers, for many of which the theoretical limits are not practical because of already existing background levels. For exexample, about $5 \times 10^3$ curies of tritium would be needed to double the 1972 background (*8*) of ~2 pc of HT per cubic meter of air after a dilution similar to that illustrated in this report. (ii) The small amounts needed and the nontoxicity of methane and its homologs should make possible their use even in populated areas. (iii) Isotopically labeled methane molecules may be useful in defining better the fate of natural methane and its role in the ozone destruction reactions of the upper atmosphere.

At the extreme sensitivities illustrated in this report, present-day techniques require the sampling of about 1 $m^3$ of air (containing about 1 $cm^3$ of methane), isolation of the methane, and determination of the mass-21/mass-16 ratio in an appropriate spectrometer. Consideration should, of course, be given to automation and simplification of the sample-collecting and measurement techniques to make experiments with this new tracer even more attractive.

The background in a spectrometer at mass 20 is typically higher by about a factor of 10 than at mass 21, but it would probably be economical to use a larger amount of methane-20 ($^{12}CD_4$) than methane-21 as a tracer since the former is less expensive to produce. If, in fact, both methanes prove to be feasible long-range tracers, it would be more effective to use them together. The cost of collection and analysis will be the critical items, and the amount of information and confidence in the data obtained can be greatly increased by the simultaneous use of methane-20 and methane-21, released at slightly different times or locations, at a relatively small increment in cost. At prices currently quoted for the sale to the public of $^{13}$CO, the starting material for the synthesis of methane-21, the cost of the separated $^{13}$C isotope in this experiment (about 50 g) was less than $5000; similar amounts are esimated for the cost of a kilogram of $^{12}CD_4$ if produced on an appropriate scale, since the cost of the special isotope in this case is relatively small and most of the expense would be for synthesis.

The release of 1 kg of methane-21 would, if uniformly mixed in the atmosphere, raise the mass-21/mass-16 ratio of atmospheric methane to $2 \times 10^{-13}$. Thus, the release of a few kilograms of methane-21 per year in tracer experiments would not add a measurable increment to the present detectability limit which is determined by the noise level in the mass spectrometer. Instrumental improvements can be introduced which should markedly improve the detection sensitivity, possibly by more than an order of magnitude, in which case tracer experiments on a global scale involving nonradioactive substances should become feasible. The relatively short life of methane in the atmosphere would prevent long-term isotopic contamination of atmospheric methane as a result of such tracer experiments.

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9. We thank R. and H. Bench of Airco, Inc., for separating the methane samples; W. Maeck of Allied Chemical Corporation for arranging and supervising the methane-21 release at the National Reactor Testing Station, Idaho; the Defense Advanced Research Projects Agency (DARPA) for permitting use of DARPA instrumentation; and Headquarters, U.S. Air Force–Air Force Technical Applications Center, for developmental and operational support. Work at Los Alamos Scientific Laboratory and at the University of Chicago was carried out under the auspices of the Energy Research and Development Administration.

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# Angiotensin-Converting Enzyme: Vascular Endothelial Localization

*Abstract.* *Fluorescein-labeled antibody to rabbit pulmonary angiotensin-converting enzyme localized in the vascular endothelium of rabbit lung, liver, adrenal cortex, pancreas, kidney, and spleen. Epithelial cells of the renal proximal tubules were the only parenchymal cells among the organs studied that demonstrated immunoreactivity.*

Angiotensin-converting enzyme catalyzes the release of histidylleucine from the COOH-terminus of angiotensin I to yield a pressor octapeptide, angiotensin II (*1*), the vasoactive agent of the renin-angiotensin system (*2*). The same enzyme inactivates bradykinin, a vasodepressor nonapeptide, by catalyzing the cleavage of its

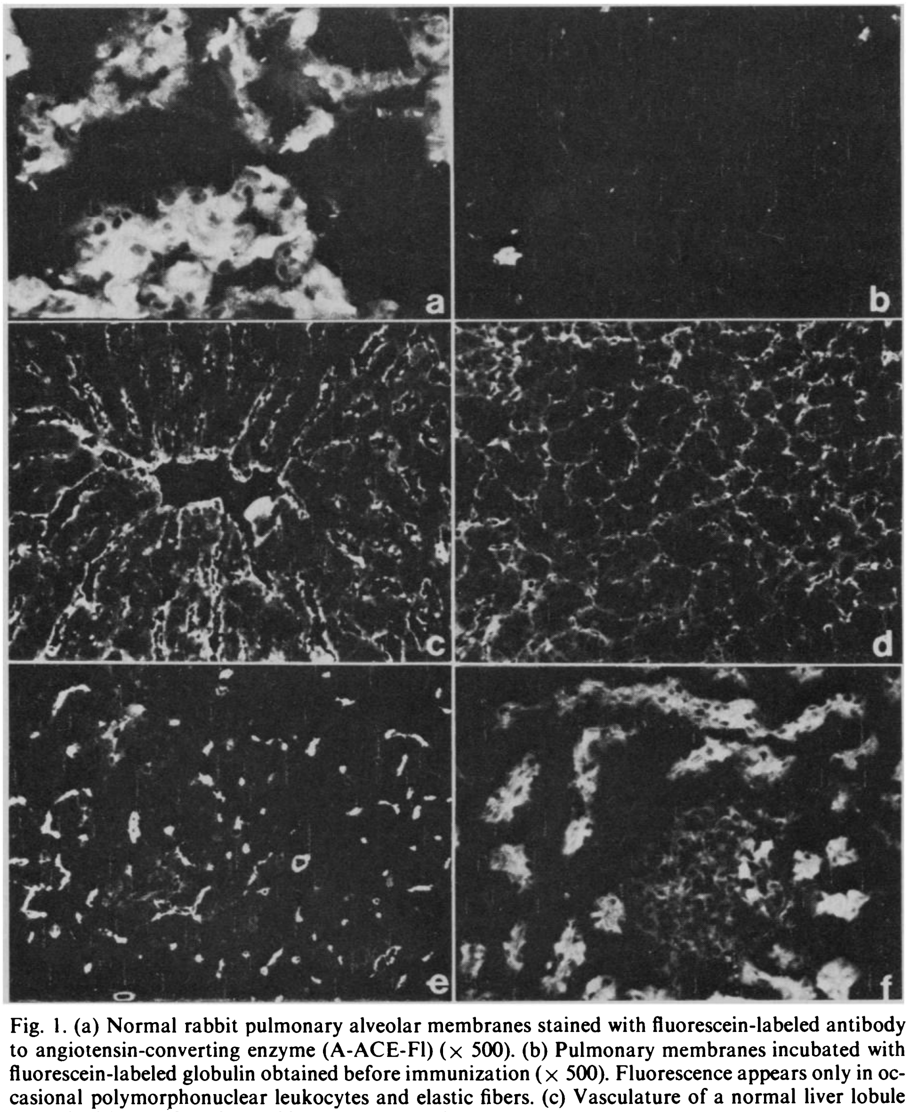

Fig. 1. (a) Normal rabbit pulmonary alveolar membranes stained with fluorescein-labeled antibody to angiotensin-converting enzyme (A-ACE-Fl) (× 500). (b) Pulmonary membranes incubated with fluorescein-labeled globulin obtained before immunization (× 500). Fluorescence appears only in occasional polymorphonuclear leukocytes and elastic fibers. (c) Vasculature of a normal liver lobule stained with A-ACE-Fl (× 100). Hepatocytes do not stain. (d) Vessel network of normal adrenal cortex stained with A-ACE-Fl (× 200). Parenchymal cells do not stain. (e) Vasculature of normal pancreas stained with A-ACE-Fl (× 100). Parenchymal cells do not stain. (f) Normal kidney showing staining both in the glomerular tuft (slight) and in the proximal tubules (prominent) (× 250).