Carbon Monoxide Production by a Bathypelagic Siphonophore

George V. Pickwell; Eric G. Barham; John W. Wilton

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Table 1. Body burdens of cesium-137 (in nanocuries) in permanent Eskimo residents of Alaskan Eskimo villages.

Adults*				Average for children†
Average		Maximum		
1962	1963	1962	1963	1963
		Anaktuvuk		
421	628	790	1240	217
		Kotzebue		
138	140	518	732	42
		Barrow		
52	60	166	177	19
		Point Hope		
17	39	119	88	17
		Fort Yukon		
	34		181	13

* 15 years or older. † 5 to 14 years.

increases that ranged from 5 to 112 percent. The average percentage increase was less than that shown by people counted at Richland, Washington, who showed an average of 6 nanocuries in 1962 and 12 nanocuries in 1963. The food chains controlling the Cs^{137} of the people in Washington are, of course, much different from those for the Eskimos. A man from Anaktuvuk Pass had the highest amount of Cs^{137} found, 1.24 microcuries.

The International Committee on Radiological Protection (ICRP) recommends that the maximum permissible body burden of Cs^{137} be set at 3.0 μc for individuals in the population at large who are not exposed to radiation in the course of their occupation (*3*). No specific recommendation was made concerning permissible average body burdens of Cs^{137} for groups such as the Alaskan Eskimos.

$Cesium^{134}$ was again present in the Eskimos in 1963 (*4*). In the ten Eskimos with the most Cs^{137}, the Cs^{134}:Cs^{137} ratio decreased from 0.012 in 1962 to 0.0067 in 1963; the actual amount of Cs^{134} decreased by 22 percent. This indicates that the composition of the fallout was changing, presumably because of the arrival of new material from nuclear tests that started in 1961. If the new material contained no Cs^{134}, and if the Eskimos were in equlibrium with the old fallout, the amounts of Cs^{134} in the Eskimos would have decreased by 28 percent, nearly the percentage observed. The fact that Cs^{134} was picked up on an air filter at Richland, Washington, in January 1963 with a Cs^{134}:Cs^{137} ratio of 0.016 may indicate that the fallout in Alaska and in Washington came from different tests.

The change in the Cs^{134}:Cs^{137} ratio indicates that the increases found at Anaktuvuk Pass were associated with new fallout material. It is improbable that any significant part of the increases were the result of more caribou having been eaten since it is a principal item in their diet and it is relatively available at all times of the year because carcasses are stored in permafrost pits. Furthermore, those people tested for radioactivity indicated that they had eaten about the same amount of caribou in 1962–63 as in 1961–62. The large increase in the Cs^{137} in the people at Point Hope resulted largely from a poor harvest of caribou in 1962 and a good one in 1963. People at Kotzebue were tested about 2 months later in 1963 than in 1962. Since their diet contains less caribou meat in the summer, they may have eliminated some of the Cs^{137} they had accumulated; earlier testing might have shown a significant increase.

Urine samples obtained during the winter of 1962–63 through the U.S. Public Health Service hospital at Fort Yukon were examined for Cs^{137}. The results indicated an average body burden of about 120 nanocuries. This was of particular interest because the main meat in the diet came from moose, which indicated another food chain of interest. For these reasons the Athapascan Indians at Fort Yukon were examined in 1963. A period of approximately 4 months, months in which there was little caribou or moose meat in their diet, is presumed to be the cause of the difference between the 34 nanocuries observed by counting and the 120 indicated by urinalysis.

HARVEY E. PALMER, WAYNE C. HANSON
BOBBY I. GRIFFIN, DALE M. FLEMING
Hanford Laboratories, General Electric Company, Richland, Washington

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5. We thank Kasume Kasugi of the U.S. Public Health Service for use of the hospital facilities at Kotzebue and Barrow and Max G. Brewer, director of the Arctic Research Laboratory, for invaluable support in transporting equipment at Barrow and Anaktuvuk Pass and the use of the laboratory facilities. This work was performed under contract No. AT(45-1)-1350 between the Atomic Energy Commission and General Electric Company.

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Carbon Monoxide Production by a Bathypelagic Siphonophore

Abstract. *A physonectid siphonophore,* Nanomia bijuga, *associated with a vertically migrating deep scattering layer, has been observed with a gas-filled float at depths in excess of 300 meters in the sea. This implies that gas is secreted and maintained in the pneumatophore against a diffusion gradient of 30 atmospheres or more. Analysis of the enclosed gases revealed concentrations of carbon monoxide exceeding 90 percent. Necessary voiding of this gas during a vertical ascent of 300 meters could give rise to a transient population of bubbles which would act as sound-reflecting targets.*

Deep scattering layers (DSL) are zones of sonic reverberation occurring at depths of several hundred meters throughout much of the ocean. A component of each layer usually migrates vertically each day, rising toward the surface at sunset and descending again before sunrise. Current theory holds that a major source of acoustic scattering from such migratory layers is resonant gas bubbles, for example, the swim bladders of fishes (*1*). Another important source recently suggested is the gas-filled floats (pneumatophores) of siphonophores (*2*) (Fig. 1), which are colonial coelenterates. The physonectid type consists of a highly contractile stolon, which bears an apical pneumatophore followed by a cluster of swimming bells and linearly arranged polyps modified for capturing and consuming prey and for sexual reproduction.

One species, *Nanomia bijuga* (*3*), was observed directly from the U.S. Navy bathyscaph, *Trieste*, at the exact levels of the deep scattering layers during dives in the San Diego Trough (*2*). These observations showed that nanomians are capable of vigorous and rapid swimming. The hauls from our nets indicate that these organisms may move vertically some 300 m in an hour or less. To keep the pneumatophore inflated at the lower end of its vertical range, its gas content must be maintained against a diffusion gradient of some 30 atm (*4*). We have attempted to learn the nature of the gases enclosed and how they are maintained under these extraordinary conditions.

The organism (colony) becomes badly fragmented when captured by our automatic opening-closing net even when

the net is towed at a speed of approximately 3.7 km/hr (2 knots). The pneumatophores, however, float to the surface of the collecting bucket and can be readily picked out with forceps. Our observations are based solely on these isolated individuals. Measurements with an ocular micrometer in a dissecting microscope at × 50 gave an estimated volume range of 0.14 to 0.46 mm^3 for the floats.

Gases contained in individual floats were analyzed by a microvolumetric technique capable of analyzing a fraction of a cubic millimeter of gas with an accuracy of ± 0.2 to 0.5 percent by volume (*5*). A solution of ammoniacal cuprous chloride was used as a carbon monoxide absorber after carbon dioxide was absorbed by an alkaline solution of sodium citrate and oxygen by the Fieser reagent. Representative figures from a total of 85 analyses made on two cruises during July 1963 off the southern California coast are given in Table 1. Values for carbon monoxide have been corrected for reagent blanks run with air. Reliability of oxygen values was constantly substantiated by checks on air which commonly gave duplicate readings for oxygen within 20.6 to 20.9 percent. All reported analyses were performed on board ship within 1 hour after collection of the pneumatophores.

Production of carbon monoxide appears to be confined to the cells of the gas gland located in the basal portion of the pneumatophore (*6*). Jacobs (*7*) reported that in this region during gas secretion minute bubbles form, coalesce, and ultimately join the larger gas phase of the float. Aboard ship we have observed this phenomenon in fresh material. The bubbles, representing newly secreted gas, form gradually over a period of 30 minutes to an hour or more and not abruptly as might be expected had the gas gland cavitated upon abrupt evacuation of the gas phase.

Fig. 1. Buoyant individuals (pneumatophores) of siphonophore colonies (*Nanomia bijuga*, about × 7). [John H. Sneed, U.S. Navy Electronics Laboratory]

Table 1. Gas content of individual floats of *Nanomia bijuga*.

Date (July 1963)	Time (hr)	Mean depth of net (m)	O_2 (%)	CO (%)	N_2* (%)	Calculated partial pressure of CO† (atm)
29	2100	60	4.5	88.3	7.2	6.2
29	2100	60	5.3	79.7	15.0	5.6
1	2200	70	3.0	86.7	10.3	6.9
1	2200	70	1.7	93.4	4.9	7.5
2	2200	95	1.7	92.7	5.6	9.7
2	2200	95	1.5	94.1	4.4	9.9
2	2200	95	1.8	88.7	9.5	9.3
29	2230	105	1.4	92.9	5.7	10.7
1	2100	110	2.9	90.2	6.9	10.8
1	2100	110	1.9	90.2	7.9	10.8
3	0800	200	2.0	85.1	12.9	17.9
3	0800	200	3.7	77.0	19.3	16.2
2	1400	250	2.8	84.2	13.0	21.8
29	1430	365	1.7	92.3	6.0	34.6
29	1430	365	2.4	86.5	11.1	32.4

* Nitrogen values were obtained by difference. CO_2 in all cases was absent or below the accuracy of the analytical method which is approximately 0.5 percent for CO_2. † Partial pressure values include the 1 atm at the surface of the sea.

As a test for secretion of new gas, several floats were relieved of nearly all of their gas phase by vacuum extraction in a 10-cm^3 syringe. Release of gas in this manner is possible due to the presence of an apical pore surrounded by a sphincter muscle. The syringe containing 1 or 2 ml of sea water and the deflated floats was set aside in a water bath. Generally, within an hour, several of the pneumatophores would be afloat. Analysis of the gas from three such floats is presented in Table 2. Whether the CO_2 values shown represent the end product of cellular respiration or are associated with CO secretion is not known.

The possibility of production of carbon monoxide by symbiotic algae must also be considered. Cultures of blue-green algae produce CO (*8*), and algal symbionts occur in some pelagic siphonophores (*9*). Many of our nanomians, however, were collected at or below the limit of penetration of daylight suitable for photosynthesis in the sea, and we could not detect such algae in microscopic sections of the pneumatophore. Furthermore, Wittenberg (*10*) was unable to demonstrate CO production by sections of the float walls of *Physalia*, a surface-dwelling siphonophore, where algal symbionts would most likely be present. Therefore it appears that the carbon monoxide of *Nanomia* is produced only by the cells of the gas gland.

Several algae and a few higher plants have been reported to produce CO in concentrations which may reach 12 percent in the case of the brown alga *Nereocystis* (*11*). The Portuguese man-of-war, *Physalia*, produces CO in concentrations occasionally reaching 20 percent (*10*). The partial pressure of CO does not go higher than 0.2 atm in either of these two species, but at a depth of 300 m *Nanomia* must tolerate a partial pressure of carbon monoxide equivalent to nearly 30 atm. We believe these are the highest concentrations of carbon monoxide yet reported as occurring naturally in a living system.

Studies of the chemical and physical mechanisms responsible for CO production, concentration, and tolerance may provide rewarding answers. A further interesting aspect is that upward migration undoubtedly results in the release through the pore of bubbles from the

Table 2. Gas content of pneumatophores after secretion of new gas.

Float	CO_2 (%)	O_2* (%)	CO (%)	N_2† (%)
A	3.0	0.6	82.9	13.5
B			73.7	26.3
C	2.0	0.5	76.2	21.3

* O_2 values are low compared with those of Table 1 because of partial extraction of this gas from the seawater surrounding the floats when the latter were deflated; such extraction limits the amount available for inward diffusion into the float. † Nitrogen values were obtained by difference.

expanding gases within the float. These will create additional sound-reflecting targets which may well be an added cause of the marked increase in scattering intensity observed as the scattering layers rise.

GEORGE V. PICKWELL
Scripps Institution of Oceanography, La Jolla, California

ERIC G. BARHAM
JOHN W. WILTON
U.S. Navy Electronics Laboratory, San Diego, California

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4. Total pressure of dissolved gases in the sea is taken as approximately 1 atm throughout the water column. Partial pressure of dissolved CO is taken as essentially zero. Diffusion gradient, as used in this report, refers to diffusion across the total float wall which varies in thickness generally within 20 to 50 μ.
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12. We thank Lt. R. L. McCampbell and the men of the *U.S.S. Marysville* and M. Sullivan, H. Kiner, and M. Taylor of the Navy Electronics Laboratory for assistance in obtaining data at sea. Dr. D. Jameson provided facilities at San Diego State College for histological sectioning. Drs. T. Enns, E. Hemmingsen, and P. Hansen gave helpful advice and discussion, and P. F. Scholander, E. Douglas, G. Curl, and E. C. Buffington critically read the manuscript. Contribution from Scripps Institution of Oceanography.

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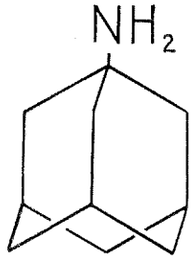
Antiviral Activity of 1-Adamantanamine (Amantadine)

Abstract. *1-Adamantanamine (amantadine) causes a selective, reproducible, dose-related inhibition of influenza infections in tissue culture, chick embryos, and mice. The compound is not virucidal and appears to act by interfering with the penetration of the host cell by the virus. In influenza infections of mice, greatest efficacy occurs with treatment at the time of infection; however, there is significant antiviral activity with treatment delayed up to 72 hours after infection. Virus inhibition is not complete and survivors are immune to a challenge infection with the original infecting virus.*

1-Adamantanamine (amantadine), a stable, colorless, crystalline amine with an unusual symmetrical structure,



reproducibly and selectively inhibits influenza viruses in tissue culture, chick embryos, and mice. The compound studied is the water-soluble salt, amantadine hydrochloride.

Most of the tissue culture studies were carried out by plaque-inhibition techniques similar to those described (*1*), although hemagglutination inhibition and hemadsorption inhibition methods were also used. Amantadine hydrochloride inhibited virus multiplication of four strains of influenza A, one of A′, three of A-2, one of C, and the Sendai strain of parainfluenza. Certain other myxoviruses were resistant, including two strains of influenza B, Newcastle disease virus, mumps, and parainfluenza strains 1, 2, and 3. A variety of RNA and DNA viruses were also insensitive. Amantadine hydrochloride showed a dose-responsive relationship in its effect on sensitive influenza strains propagated in chick fibroblasts with a maximum inhibition of 25 μg per milliliter and significant inhibition at 1 to 2 μg per milliliter. In one-step multiplication cycle experiments the maximum effect of the compound was a one-log drop in virus production. No toxicity to chick-embryo fibroblasts was seen at 30 μg per milliliter; at 100 μg per milliliter toxicity was manifested only by retardation of cell growth rate. Antiviral activity was also demonstrated in cultures of monkey kidney cells, canine kidney cells and human amnion cells.

Table 1. Length of treatment and effect of specific antiserum on the antiviral activity of amantadine hydrochloride against influenza A-2, Jap. 305.

Amantadine-HCl (20 μg/ml)	Duration of treatment (hr)	Specific antiserum*	Virus increase at 24 hours --(− log HA titer)
−	−	−	2.29
+	2 †	−	1.77
+	24 ‡	−	1.21
−	−	+	1.90
+	2 †	+	1.08

* Added 1.75 hours after virus. Cultures were washed 2 hours after virus and incubated at 37°C for 24 hours. † Compound added 15 minutes before virus. Cultures were washed 2 hours after virus to remove compound and incubated at 37°C for 24 hours. ‡ Added 15 minutes before virus and present for whole incubation period.

Amantadine hydrochloride caused no inactivation of viruses held in contact with concentrations of 1 to 25 μg per milliliter for 24 hours at 37°C; at 100 μg or more per milliliter, a number of viruses were inactivated, including some whose multiplication in tissue culture was not inhibited by 25 μg per milliliter. Thus direct inactivation of virus by amantadine hydrochloride is not a major factor in the activity of the compound in tissue culture. The adsorption of influenza A to chick embryo fibroblasts and red blood cells was not affected by 25 μg of amantadine hydrochloride per milliliter nor was the enzymatic release of virus from red blood cells affected by the compound.

Preliminary tissue culture studies have indicated that amantadine hydrochloride blocks or slows penetration of the host cells by the virus. The evidence for this is as follows. Cultures of chick embryo cells were infected with a multiplicity of 20 so that all cells were infected at the beginning of the experiment. To obtain high antiviral activity amantadine hydrochloride had to be present within 5 minutes with cells and virus held at 37°C and within 60 minutes with cells and virus held at 23°C. All cultures were incubated for 24 hours at 37°C immediately after adding compound, and after 24 hours virus production was measured by hemagglutinin titration on cells and culture fluid. Similar results were obtained with homologous antiserum under identical conditions. Partial reversal of the antiviral activity of amantadine hydrochloride was accomplished by washing the infected cell layers during the incubation period. The greatest antiviral activity occurred when the compound was present during the entire period of incubation.