activity in the enriched and control light tubes and the agreement between the results from the bioassay tubes and the river support our conclusion that the differences are real.
12. The method is essentially that of K. Gocke [*Mar. Biol.* **42**, 131 (1977)] modified for use with undisturbed epilithon. Colonized glass beads were incubated with filtered water from the appropriate bioassay tube containing 0.5 μg/liter ^{14}C-labeled substrate. Assimilation was measured as incorporation of ^{14}C into bacterial biomass on the beads. Respiration was measured as $^{14}CO_2$ released upon acidification. Turnover times (T_t) were calculated by the following equation: $T_t = t/f$ where t is incubation time (hours) and f is (^{14}C uptake + respiration)/(^{14}C added).
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22. After 23 days of enrichment, blackflies were collected from riffles upstream and 340 m downstream of the enrichment sites by washing randomly selected rocks. Blackflies were subsampled from each preserved collection by randomly selecting approximately 100 larvae from a gridded petri dish and measuring body lengths. Larvae from the enriched riffle were significantly larger than those from the upstream riffle ($t(202) = 5.50$, $P < 0.005$). Baseline data from 1978 showed that blackflies from different riffles in the unenriched stream did not differ significantly in body length.
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24. During 1978, 1979, and 1980, B.J.P. and J.E.H. measured carbon flows in the undisturbed river. Net primary production of the epilithic community was 13 g of carbon per square meter per year. Inputs of eroding peat were 200 to 300 g/m^2/year^1 and inputs of DOC were roughly ten times greater than peat inputs. Thus inputs of organic matter from the tundra vegetation were about 100 times greater than primary production in the river and much of the metabolism of the stream biota depended on the utilization of this organic matter from the tundra. This is supported by the data in Fig. 2A that shows that metabolic heat production of epilithic communities which developed in the dark without added nutrient is roughly 70 percent of the activity in the light, which would include algal metabolism as well.
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Increase of Atmospheric Methane Recorded in Antarctic Ice Core

Abstract. *Air entrapped in bubbles of cold ice has essentially the same composition as that of the atmosphere at the time of bubble formation. Measurements of the methane concentration in air extracted by two different methods from ice samples from Siple Station in western Antarctica allow the reconstruction of the history of the increase of the atmospheric methane during the past 200 years.*

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The atmospheric methane concentration is increasing at a rate of about 1.2 to 1.9 percent per year (*1*). This increase is caused by an increase of the emission rate and possibly by a depletion of the concentration of OH radical in the atmosphere. The reaction of these radicals with methane in the gas phase is the dominant sink and determines the atmospheric lifetime. A higher concentration of CH_4 causes a higher temperature on the earth surface due to an increased greenhouse effect of the atmosphere (*2*). Measurements on air extracted from air bubbles of polar ice cores make it possible to investigate the atmospheric CH_4 concentrations of the past. On the basis of such measurements, Craig and Chou (*3*) and Rasmussen and Khalil (*4, 5*) reported a constant atmospheric CH_4 concentration of about half the present value until about 300 years ago. Younger samples indicate an increase to the present value.

We report measurements made on samples from an ice core from Siple Station in Antarctica. These samples largely fill the gap between previous measurements on ice samples and direct measurements on atmospheric air. Samples from Siple Station are well suited for investigating changes of the atmospheric composition in the recent past, having an excellent time resolution because of the high accumulation rate.

During the formation of ice, air is separated from the atmosphere and enclosed in bubbles. At locations with a low mean annual air temperature (<−20°C), ice is formed by sintering of dry cold firn without interaction with meltwater. At Siple Station (75°55′S; 83°55′W) the mean annual air temperature is −24°C and the mean annual accumulation rate is 500 kg m^{-2}. An ice core was drilled in the Antarctic summer 1983–1984 to a depth of 200 m by the Polar Ice Coring Office (Nebraska) and our institute. It is possible to date the ice to an accuracy of about 2 years down to a depth of 144 m by counting seasonal variations of the electrical conductivity.

The enclosure process of air was investigated by measuring open and isolated porosity on several samples (*6*). The enclosure occurs between 64 and 76 m below surface. Assuming that the air is well mixed in the permeable part of the firn down to the transition from firn to ice, the difference between the mean gas age and the age of the surrounding ice is 95 years, and the time interval needed for gas enclosure is about 22 years. Narrow impermeable layers observed at a depth of 68 m and below are, however, already sealing the air below from the atmosphere. This was also indicated by measurements of the CO_2 concentration on the same ice core (*7*). When this effect is taken into account, the difference between the mean gas age and the age of the surrounding ice is estimated to be about 80 to 85 years instead of 95 years.

Air is extracted from the bubbles of ice samples by a melt extraction and a dry extraction technique. For the vacuum melt extraction, ice samples of about 400 g, containing about 10 percent by volume of air, are melted in an evacuated glass container. The escaping gas is collected continuously during the melting process with a Toepler pump into a small glass bulb (*8*). The advantages of the melt extraction method are the extraction efficiency of almost 100 percent and the simplicity. The disadvantage is the presence of water, where production or consumption of methane by microbiological

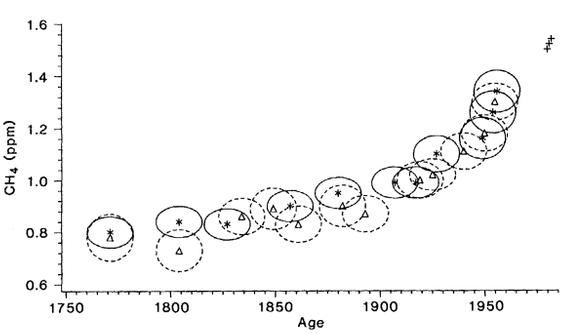

Fig. 1. Measured CH_4 concentration plotted against the estimated mean gas age. Stars with solid ellipses represent results obtained from melt extraction, and triangles with dashed ellipses represent results from dry extraction. The plus signs indicate measurements on atmospheric air (*1*). The vertical semiaxes of the ellipses indicate the estimated precision, and the horizontal semiaxes the duration of the gas enclosure process.

activity or interaction with chemical impurities could occur.

For the dry extraction method, ice samples of about 600 g are ground in an evacuated steel container with a milling cutter. The air escaping from the mechanically opened bubbles is collected by condensation at 14 K in a small steel cylinder. The milling cutter is driven by a magnetic coupling with the driving part outside the evacuated chamber, so that any dynamic vacuum sealing can be avoided. All parts that are in contact with the ice sample or the extracted gas are stainless steel. The crushing device is operated in a cold room at −20°C (*9*). There is no meltwater involved and therefore no danger of any reactions with the liquid phase that might lead to a gain or loss of gas components. The extraction efficiency is 80 to 90 percent since not all bubbles are opened.

The blank values of both extraction methods were determined by performing gas extractions with pure single-crystal ice samples. Either a small amount of CH_4-free nitrogen or of a standard gas mixture is added into the evacuated container before the single crystal is melted or ground. About the same amount of gas is added as is normally enclosed in a natural ice sample. The tests for the melt extraction give a contamination between 0.05 and 0.09 parts per million (ppm) by volume. Measurements are corrected with the blank value obtained by measuring a single-crystal ice sample immediately before the sample is measured. The contamination for the dry extraction method is between 0.21 and 0.3 ppm by volume. The blank value for our dry extraction system is rather high. The contamination with methane increases linearly with the number of revolutions of the milling cutter that are needed to grind the sample. Friction between metals is most probably the cause of the contamination. The contamination of each sample was calculated, taking into account the number of revolutions to grind the sample. The uncertainty of the calculated contamination is estimated to be less than 0.05 ppm.

The extracted gas is measured with the flame ionization detector of a gas chromatograph (Hewlett-Packard 5880A). For one analysis 0.6 to 1.5 ml of sample gas is needed at standard temperature and pressure. As standard gas, two mixtures of N_2, O_2, Ar, and CO_2 in atmospheric composition are used, and 0.98 ± 0.03 or 3.12 ± 0.09 ppm of CH_4, respectively (Messer Griesheim). The separation column is filled with Carbosieve S-II (Supelco S.A.). Helium is used as a carrier gas.

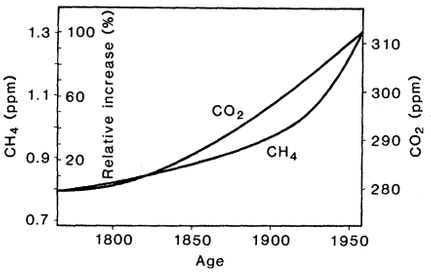

Fig. 2. Comparison of the increase of atmospheric CO_2 and CH_4 concentrations determined by measurements on ice samples from Siple Station.

From the Siple ice core, 11 ice samples were measured by vacuum melt extraction and 12 by dry extraction. Two analyses were performed for each sample gained with the melt extraction method and three to four for each sample with the dry extraction method (Table 1 and Fig. 1). Results from measurements of ice core samples from the South Pole are also shown in Table 1. They represent samples with an older mean gas age.

The vertical semiaxes of the ellipses in Fig. 1 are given by the estimated precision of the measurements. The estimate includes the uncertainty of the contamination correction as well as the 1 standard error of the mean of the analyses made with the gas chromatograph. A possible inaccuracy of the standard gas of 3 percent is not included.

Table 1. Methane concentration (in parts per million by volume) in air extracted from ice samples. The error limits are the estimated precisions of the measurements.

Depth (m)	Gas age (A.D.)	Methane (ppm)
	Siple Station	
Dry extraction		
78.6	1955	1.30 ± 0.07
82.6	1950	1.18 ± 0.07
88.0	1940	1.11 ± 0.07
96.4	1925	1.02 ± 0.06
100	1919	1.00 ± 0.07
113	1893	0.87 ± 0.07
119	1882	0.90 ± 0.08
130	1861	0.83 ± 0.07
136	1849	0.89 ± 0.08
144	1834	0.86 ± 0.07
160	1804	0.73 ± 0.08
177	1771	0.78 ± 0.09
Vacuum melt extraction		
78.0	1956	1.34 ± 0.08
80.0	1954	1.26 ± 0.08
83.0	1949	1.16 ± 0.08
96.0	1927	1.10 ± 0.07
101	1917	0.99 ± 0.06
107	1907	0.99 ± 0.06
120	1880	0.95 ± 0.06
132	1857	0.90 ± 0.06
147	1827	0.83 ± 0.06
160	1804	0.84 ± 0.06
177	1771	0.80 ± 0.06
	South Pole	
153	1630	0.84 ± 0.06
154	1624	0.84 ± 0.11
215	1	0.73 ± 0.11
216	1	0.66 ± 0.08

The mean gas age is calculated by subtracting 82 years from the age of the ice (*7*). The horizontal axes of the ellipses (Fig. 1) indicate the duration of the gas enclosure process and do not include an uncertainty of the calculated mean gas age.

The measurements made with both extraction methods give the same results within the error limits. No significant loss or production of methane due to the presence of water is observed. We conclude therefore that both extraction methods are practical.

Because gas enclosure occurs rapidly at Siple Station, we can measure young air samples. The youngest one represents an age range between about 1945 and 1965. The present atmospheric CH_4 level is well within the range of the concentration that can be extrapolated from our ice data. The results do not give any indication that concentrations of CH_4 around 1950 were similar to those at present as Ehalt *et al.* (*10*) proposed on the basis of infrared spectroscopic measurements.

To compare our results with results obtained by Rasmussen and Khalil with ice samples from Camp Century (77°11′N, 61°09′W), Crête (71°07′N, 37°19′W), and Byrd Station (79°59′S, 120°01′W) (*5*), we had to recalculate the age of the enclosed gas. Rasmussen and Khalil used as a first approximation a mean age difference of enclosed air and surrounding ice of 90 years for all stations. According to Schwander and Stauffer (*6*), however, this difference is 130 years for ice from Camp Century, 200 years for Crête, and 240 years for Byrd Station. When the corrected ages are used, their results agree with our results from Siple Station within the error limits.

In Fig. 2 the increase of the atmospheric methane concentration is compared with the increase of the atmospheric CO_2 concentration, which was measured on the same ice core from Siple Station (*7*). It has to be kept in mind that the relation between the concentration and the production rate is different for CO_2 and CH_4 because of a different system behavior. The increase of CH_4, relative to the total increase from 1760 until 1960, was smaller until about 1915 than the corresponding CO_2 increase but larger afterwards. An increasing CO_2 input parallels an increasing input of CO into the atmosphere, especially if the CO_2 originates from

biomass burning (*11*). An increased CO concentration on the other hand was suggested as a reason for a decrease of OH radicals in the atmosphere (*12*). A decrease of OH radicals, the main sink for CH_4 (*13*), is therefore leading to an increase of the CH_4 concentration (*5*). The rapid increase of the CH_4 concentration may therefore be caused in part by an increase of the CO concentration.

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13. The question arises whether OH radicals could be responsible for a depletion of CH_4 in the air bubbles after enclosure. A possible source for OH radicals is H_2O_2 dissolved in the ice and diffusing slowly into the air bubbles. Different concentrations of H_2O_2 in ice from different locations have been measured by A. Neftel, P. Jacob, and D. Klockow [*Nature (London)* **311**, 43 (1984)]. The influence of such a reaction in natural ice would be small. Whether the suggested reaction occurs at all in natural ice is not yet known.

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Transcription of Novel Open Reading Frames of AIDS Retrovirus During Infection of Lymphocytes

Abstract. *The retrovirus frequently isolated from patients with the acquired immune deficiency syndrome (AIDS) has two novel open reading frames previously designated "A" and "B." The "A" region was found to be specifically expressed as polyadenylated RNA's of 5.5 and 5.0 kilobases in infected cells. The "B" region was expressed as 1.8- to 2.0-kilobase RNA species. Additional full-length and spliced messenger RNA's of the* env *region were also identified.*

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A novel human retrovirus has been isolated from patients with acquired immune deficiency syndrome (AIDS) and the AIDS-related complex (ARC) (*1*). In vitro, this retrovirus is cytopathic for human T-cells of the OKT4/Leu-3 phenotype, the same cells that are selectively depleted in AIDS patients. This effect on lymphocytes, coupled with seroepidemiologic data linking infection by the AIDS retrovirus (AIDS RV) with the occurrence of disease, has implicated the AIDS RV as the etiologic agent of AIDS. A crucial question in understanding the pathogenesis of AIDS is the mechanism by which the AIDS RV specifically infects and kills lymphocytes. In addition to the *gag*, *pol*, and *env* genes, the AIDS RV genome contains two unique open reading frames (*2, 3*) which we have previously called "A" and "B" (*4*). In this report we show that both the "A" and "B" genes are transcribed as spliced subgenomic RNA's in cells infected by the AIDS RV.

We have used infected normal human lymphocytes stimulated by phytohemagglutinin (PHA), as well as a continuous human T-cell line, A3.01 (*5*), to study the transcription of viral RNA during cytolytic infection. AIDS RV infection of A3.01 cells mimics that seen in stimulated lymphocytes; reverse transcriptase (RT) activity can be detected in culture supernatants several days after infection, and cell death follows shortly thereafter. We have also examined AIDS RV transcription in the continuous virus producer line H9 (*6*).

Polyadenylated [poly(A)$^+$] RNA was prepared from acutely infected PHA-stimulated lymphocytes or A3.01 cells and from virus producing H9 cells. Infected PHA-stimulated lymphocytes and A3.01 cells were harvested after the onset of viral cytopathic effect (syncytia formation) but before the appearance of RT activity. Infected cell RNA preparations were then examined by Northern blot hybridization with the use of molecularly cloned segments of the AIDS RV DNA as probes (Fig. 1). The long terminal repeat (LTR) (probe 1), *gag* (probe 2), *pol* (probe 3), *pol*-"A" (probe 4), and *env* (probe 5) DNA segments were isolated by restriction enzyme cleavage of plasmid subclones of the lymphadenopathy virus (LAV) proviral DNA (*7*). Two 30-bp oligonucleotide probes, specific for the novel open reading frames of the AIDS RV, were synthesized according to the published sequences of LAV and ARV-2 (*2*). In this report we use a nucleotide numbering system based on the sequence of a complete copy of LAV proviral DNA. To convert nucleotide positions to those of the AIDS RV RNA genome, one should subtract the number of nucleotides present in the U3 LTR: 456 bp for LAV, 453 bp for HTLV-III and ARV-2 (*2, 3*). The "A" region oligomer (5562–5591) was a perfect match (30/30 bp) with both the LAV and ARV-2 sequences. The synthetic "B" region probe (8892–8921) was a 30/30-bp match with the LAV sequence and a 29/30-bp match with ARV-2.

Figure 2 shows Northern blot hybridizations of poly(A)$^+$ RNA from cells infected with AIDS RV. In these experiments, stimulated human lymphocytes (lanes a to c) or A3.01 cells (lanes d to k) were infected with virus isolated from an AIDS patient living in New York. This isolate, NY-5, has biological and biochemical properties similar to other AIDS RV's; a molecular clone of NY-5 proviral DNA contains a number of restriction sites present in several other viral isolates (*8*). Since all retroviral messenger RNA's (mRNA's) have LTR sequences at their 5′ and 3′ ends, the complete array of AIDS RV transcripts present in an infected cell should hybridize to an LTR probe (probe 1). As shown in Fig. 2, lane a, five discrete LTR-reactive bands were present in infected stimulated lymphocytes. These included the full-length, 9.1-kb viral genomic RNA and comigrating *gag*/*pol* mRNA's; four subgenomic mRNA species of 5.5, 5.0, 4.3, and 1.8 to 2.0 kb in size were also seen. Similar LTR-reactive transcripts were also observed in A3.01 cells infected with NY-5 (lane d). No hybridization of the LTR probe to RNA prepared from uninfected stimulated lymphocytes