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Long-Term Effects of Crude Oil on Uptake and Respiration of Glucose and Glutamate in Arctic and Subarctic Marine Sediments†

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The effects of crude oil on uptake and respiration (mineralization) of glucose and glutamate in marine sediments were investigated. After the sediments were treated with crude oil, they were replaced at or near the collection site by scuba divers. These sediments remained in situ until they were retrieved for analysis. Glucose and glutamate uptake rates were found to decrease, and the percent respired was found to increase in Arctic and subarctic marine sediments that had been exposed to fresh crude oil. These same changes were also observed when "weathered" crude oil was used and when untreated sediments were overlaid with oiled sediments. When the kinetics of glutamate uptake were determined, both the maximum potential uptake rate and the turnover time were significantly affected. A comparison between the proportion of glucose taken into the cells and that respired as CO_2 indicated that crude oil affected biosynthetic mechanisms. A study of sediments that had been exposed to crude oil for at least 5 months showed that glutamate transport into the cells was affected more extensively than biosynthetic mechanisms. In the initial months of exposure, bacterial concentrations and total adenylate concentrations were found to decrease in the presence of crude oil. Our data suggest that secondary productivity in the marine environment could be adversely affected by the presence of crude oil in marine sediments.

With the increased production and transport of crude oil along the Alaskan coast, there is increasing concern about the potential impact of a large oil spill on the inshore marine environment. More specifically, there is a growing apprehension that such a spill or a series of spills may significantly reduce the productivity of the fisheries and adversely affect other wildlife in this pristine environment. There have been studies which suggest that rates of crude oil biodegradation and hydrocarbon evaporation may be reduced in cold marine waters which could effectively increase crude oil toxicity (14, 15). Sanders et al. (16) have reported that no. 2 fuel oil can persist in marine sediments for up to 5 years. These findings suggest that the toxic effects of petroleum products may persist over an extended period of time in the marine environment. While studying crude oil degradation in Beaufort Sea waters, Horowitz and Atlas (14) observed that the residual oil contained "similar percentages of individual components and classes of hydrocarbons" as that found in the original oil regardless of the degree of degradation. Thus, in Arctic marine waters, toxic components of the crude oil may persist even though there may have been significant degradation.

Increased concentrations of hydrocarbon-utilizing bacteria in marine waters and sediments exposed to crude oil have been documented (19, 22). In another study, natural marine microbial populations exposed to crude oil for 12 h or less showed a decrease in relative microbial activity when compared to untreated controls (10). In the same study it was shown that pelagic microorganisms that were exposed to crude oil for longer periods of time (up to 9 days) were apparently able to recover normal heterotrophic activity with increased incubation time. In a recent review of the literature, Colwell and Walker (6) concluded that the two major microbial responses to a crude oil spill are the increase in hydrocarbon-utilizing bacteria and the inhibition of certain other bacteria which might ultimately affect microbial processes. Even though the presence of crude oil in marine sediments should theoretically alter microbial processes, few observations of this type have been reported. Our objective in this study was to

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determine whether crude oil affects organic substrate uptake and respiration by microorganisms in arctic and subarctic marine sediments.

MATERIALS AND METHODS

Sample collection, preparation, placement, and retrieval. Sediment was collected from the bottom of Kasitsna Bay using a pipe dredge. In the Elson Lagoon (Point Barrow), Sadie Cove, and Coal Bay studies (Fig. 1 and 2), sediments were collected in buckets by scuba divers. Several sediments collected in this manner from the same location were combined into 120-liter plastic containers and thoroughly mixed. Subsamples of the resulting sediment mixture were supplemented with 400 ml of fresh crude oil for every 8 liters of sediment (50 ppt). In the Elson Lagoon study, Prudhoe Bay crude oil was used, and in the Kasitsna Bay study, Cook Inlet crude oil was used. Unsupplemented sediment served as controls. Subsamples were dispensed into six Plexiglas trays measuring 30.5 by 30.5 by 10.2 cm. In the August 1979, November 1979, and January 1980 Kasitsna Bay studies, the sediments were dispensed into 500-ml plastic jars. The trays were filled with 8 liters of sediment and lowered approximately 20 m to the bottom of the bay by scuba divers. Divers also retrieved the trays after the desired incubation period. During placement and retrieval the trays and jars were covered with plastic lids to reduce sediment washout. Because the analysis took approximately 2 days, the trays and jars were kept in a trough through which fresh seawater was circulated at a rate of approximately 8 liters/min. This kept the sediments at in situ temperature and ensured the replenishment of dissolved gases. Subsamples (cores) were removed from each tray using a 50-ml plastic syringe with the end of the barrel removed. Five of these cores were removed and combined in a plastic 500-ml jar. These combined subsamples were then homogenized by vigorous shaking and analyzed.

The seawater flow system at the laboratory consisted of a stainless-steel jet pump located 125 m offshore pumping seawater through a polyvinyl chloride plastic plumbing system. The only materials that the seawater came into contact with were polyvinyl chloride plastic, natural latex rubber tubing, nylon, and stainless steel.

Samples of sediment supplemented with various concentrations of crude oil (incubation up to 3 months) and samples of sediment supplemented with 50 ppt crude oil incubated for various periods of time (up to 21 days) were kept in 500-ml jars which were placed in a flowing seawater system. The water in this system was continually replenished with fresh seawater, and the samples were covered with approximately 5 cm of water at all times with no exposure to direct sunlight. We feel that the use of the flow system was justified in the short-term experiments for two reasons. Where

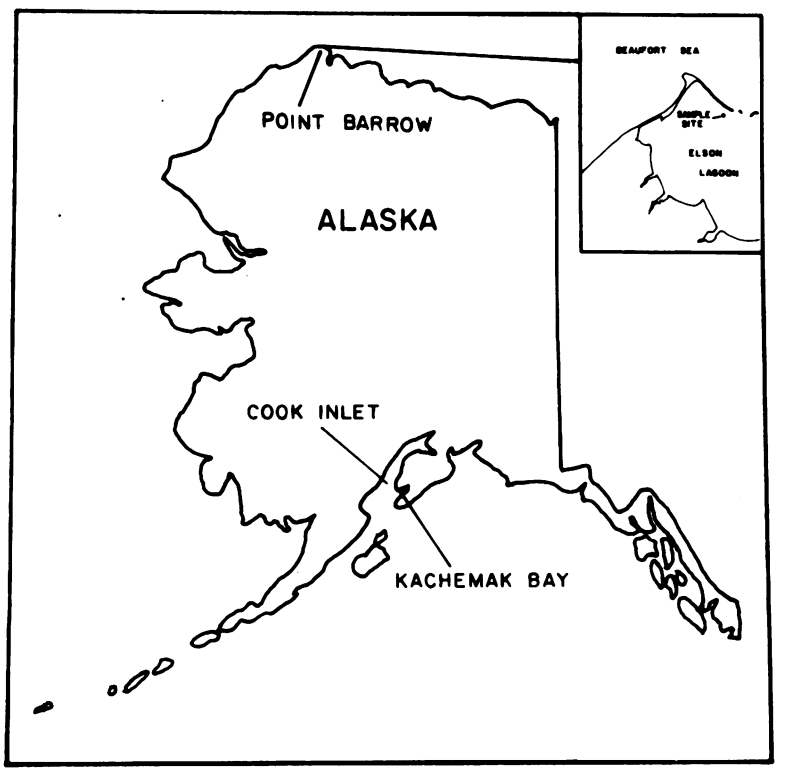

FIG. 1. *Location of the sampling areas relative to the state of Alaska. Inset shows sample site location within Elson Lagoon near Point Barrow.*

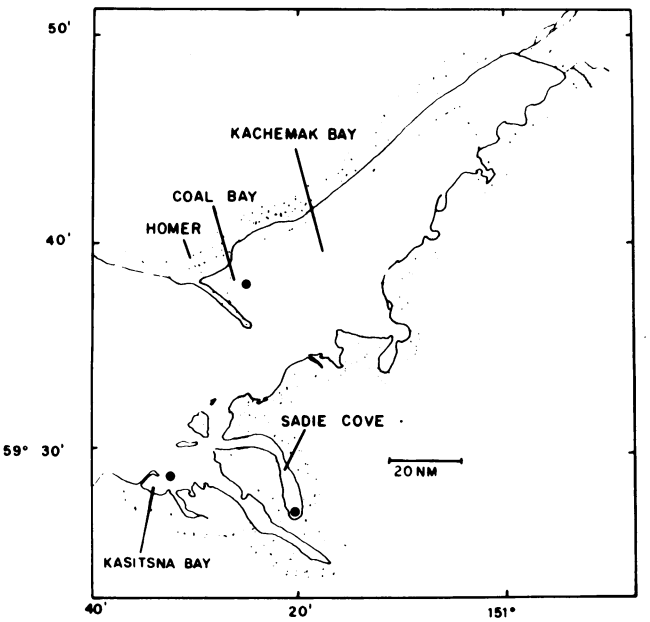

FIG. 2. *Sample site location within Kachemak Bay.*

direct comparisons could be made, the results of the experiments conducted in this way were the same as those obtained in the in situ experiments. Studies conducted in indoor aquaria fed by the same flow system produced data that were identical to those obtained in in situ tray experiments for periods up to 8 months. After that time, detritus accumulating in the aquaria apparently altered the observations made. The results of the aquaria experiments were not included in the data presented in this report.

Crude oil overlay experiments were also performed in which sediment was placed in Plexiglas trays to a depth of 9 cm. Subsamples of the same sediment were supplemented with either fresh or "weathered" crude oil, and the supplemented sediment was used to form a 1-cm layer over the untreated sediment. These trays were then placed on the bottom of Kasitsna Bay as described previously. The weathered crude oil was prepared by placing 12 liters of fresh Cook Inlet crude oil into an epoxy-coated plywood box (0.8 by 2.0 by 30 cm deep) containing seawater to a depth of 10 cm. The box and its contents were exposed outdoors for 6 weeks. At the end of the exposure time, the crude oil was reduced to a volume of 4 liters.

Heterotrophic activity. The procedures used in the uptake studies have been described by Griffiths et al. (12). The radioisotopes used were D-[*U*-^{14}C]glucose, L-[*U*-^{14}C]glutamic acid, and [*U*-^{14}C]acetate, with specific activities of 328, 10, and 57.8 mCi/mmol, respectively; all substrates were purchased from Amersham Corp. The final concentrations of substrate used in the single concentration uptake studies were 3.8, 65, and 29 μg/liter for glucose, glutamic acid, and acetate, respectively. The concentrations of glutamic acid used in the heterotrophic activity studies were 16, 32, 65, and 110 μg/liter.

The reaction vessels, containing 10 ml of a 1:1,000 sediment suspension in sterile artificial seawater, were incubated undisturbed in the dark for up to 8 h. The incubation temperature was within 1°C of the in situ temperature which varied from 2 to 11°C. In the kinetic experiments, the incubation time varied from 1 to 4 h. The calculation of the kinetic variables was made using the equations of Wright and Hobbie (20).

Adenylate concentrations. The procedure used to determine adenylate concentrations was a modification of that described by Bulleld (4). In a 50-ml beaker, 1 ml of sediment and 8 ml of extraction buffer (0.04 M Na_2HPO_4 adjusted to pH 7.70 with 0.02 M citric acid) were mixed. The sediment was dispensed using a 3-ml plastic syringe with an opening of 2 mm. The beaker was then placed in a boiling water bath for 2 min. All samples were kept cold by placing the beakers in crushed ice except during the extraction procedure. Evaporation was minimized by covering the beakers with watch glasses. A 0.1-ml volume of 10^{-6} M adenosine triphosphate was added to both sediment samples and to controls containing no sediment. The added adenosine triphosphate was used to determine the extraction efficiency. Duplicate subsamples of each sediment were extracted. After extraction, the samples were cooled in an ice bath and then added to centrifuge tubes. The beakers were rinsed with a small volume of distilled water to help recover the extracted sample. After centrifuging at 8,000 × *g* at 0°C for 10 min, the clear supernatant was decanted into a screw-capped vial, its volume was brought up to 10.0 ml with distilled water, and it was frozen at −20°C until the time of analysis.

For the analysis of adenosine triphosphate, 1.8 ml of extract was added to 0.72 ml of buffer A (0.55 g of K_2SO_4 and 1.5 g of $MgSO_4$ in 10.0 ml of distilled water)

and 0.2 ml of tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid buffer [20 mM tris-(hydroxymethyl)aminomethane-2 mM ethylenediaminetetraacetic acid, pH 7.75]. For the analysis of adenosine triphosphate plus adenosine diphosphate, 1.8 ml of extract was added to 0.72 ml of buffer B (10 ml of buffer A with 5 mg of phosphophenol pyruvate) and 0.2 ml of pyruvate kinase (Sigma Chemical Co.) solution [10 mg in 10 ml of tris(hydroxymethyl)-aminomethane-ethylenediaminetetraacetic acid]. For analysis of adenosine triphosphate plus adenosine diphosphate plus adenosine monophosphate, 1.8 ml of extract was added to 0.72 ml of buffer B, 0.2 ml of pyruvate kinase, and 0.05 ml of adenylate kinase solution (Sigma). Each mixture was held on ice during its preparation and then incubated at 30°C for 15 min and returned to the ice bath.

An adenosine triphosphate photometer and integrator-timer (American Instrument Co.) was used for the assay. A 575-μl volume of each mixture was transferred to a cuvette and placed in the photometer chamber. Integration of photon emissions over consecutive 3-s intervals was begun at the instant at which 100 μl of a buffered luciferin-luciferase preparation (Du Pont Co., no. 750145-902) was injected into the chamber through a septum. The maximum integrated count, usually occurring in the second interval, was used to calculate the concentration of adenylates in the original sample.

Acridine orange direct cell counts (AODC). In a vial, 10 ml of the 1:1,000 sediment suspension was fixed in the field laboratory by adding 1.0 ml of membrane-filtered (0.45 μm) formaldehyde (37%). These vials were sealed and shipped back to the laboratory. The cells in the sediment suspension were stained with acridine orange using the procedures of Zimmerman and Meyer-Reil (21). Where necessary, the sediment suspensions were diluted with filter-sterilized artificial seawater to keep the number of organisms per field within countable limits. Control counts were run using filtered artificial seawater with all the reagents in the normal procedure. These counts were never more than 5% of those found in the samples and were considered insignificant.

RESULTS AND DISCUSSION

Measurements of relative microbial activity as determined by labeled substrate uptake rates were conducted on subarctic marine sediments (from Kasitsna Bay) that had been exposed to 50 ppt fresh Cook Inlet crude oil for periods up to 18 months (Table 1, part A). With the exception of the 8-month exposure observations, relative microbial activity was significantly reduced in the oiled sediments regardless of the substrate used. This study was conducted on identical subsamples, with periodic measurements made at different exposure times. The same change was observed in sediments collected from various locations in Kachemak Bay at different times of the year (Table 1, parts B to F). Relative microbial activity reductions were also observed in Beaufort Sea sediments treated with 50 ppt Prudhoe Bay crude oil during a similar study conducted in Elson Lagoon near Point Barrow, AK (Table 2). Unlike the Kachemak Bay sediments, these sediments did not show consistent reductions in microbial activity until the sediments had been exposed for 13 months.

During an actual crude oil spill, it is possible for fresh, i.e., relatively unaltered, crude oil to become incorporated into marine sediments. It is more likely, however, that spilled crude oil or petroleum products will have lost many of the more volatile components before becoming associated with marine sediments. For this reason, we also measured the effects of a "weathered" crude oil on relative microbial activity (Table 3). In sediments that had been exposed to weathered crude oil at two concentrations for 1 year, there was a significant reduction in glucose uptake which was comparable to the change observed in sediments exposed to fresh crude oil.

Crude oil would probably not become thoroughly mixed into the sediments except in the intertidal zone where wave action would mix sediment with crude oil. In subtidal and littoral zones, it is more likely that crude oil would become associated with suspended matter and layered on top of the existing sediment (8). To imitate this condition, we layered 1 cm of treated sediment on 9 cm of untreated sediment and measured relative microbial activity in the entire sediment after an exposure of 1 year (Table 3). We found that, under these conditions, the fresh crude oil still significantly depressed relative microbial activity but that "weathered" crude oil did not. A glass capillary gas chromatographic analysis of the "weathered" crude oil showed essentially a complete absence of lower-molecular-weight aliphatics below nC_{13} and aromatics below dimethylnaphthalene (J. R. Payne, personal communication). The absence of these low-molecular-weight hydrocarbons may explain why no significant depression was observed in glucose uptake rates in the sediments layered with weathered crude oil-treated sediments.

Although crude oil concentrations greater than that used in this study have been reported in marine sediments after a major oil spill (18), this is probably a rare event, and oil concentrations in the 0.1-to-1.0-ppt range are more common in heavily contaminated areas (2, 3, 18). Since the concentration used in our studies was high compared to those normally encountered in highly contaminated areas, we wanted to determine if the same changes occurred at lower, more realistic crude oil concentrations. Therefore, studies were conducted using three sedi-

TABLE 1. *Effect of fresh crude oil at 50 ppt on glucose, acetate, and glutamic acid uptake rates and percentage of respiration in subarctic marine sediments*

Site of subsample	Exposure time (months)	Substrate	Substrate uptake			Respiration (%)		
			Control ($\bar{y}$)[b]	Oil ($\bar{y}$)	$P \leq$	Control ($\bar{y}$)	Oil ($\bar{y}$)	$P <$
A. Kasitsna Bay (Feb. 1979)[c]	1.5	Glucose	22	11	0.005	20	38	0.0001
		Glutamate	335	92	0.001	44	64	0.00001
	5	Glucose	77	31	0.0001	14	51	0.00001
		Glutamate	605	232	0.0001	46	50	0.01
	8	Glucose	55	34	NS[d]	16	35	0.005
		Glutamate	448	353	NS	46	49	NS
	11	Glucose	36	18	0.05	19	34	0.0005
		Glutamate	246	90	0.005	49	55	NS
		Acetate	54	14	0.05	23	38	0.005
	18	Glucose	113	65	0.05	22	40	0.001
		Glutamate	1,223	728	0.05	47	54	0.05
		Acetate	38	24	0.05	31	37	NS
B. Kasitsna Bay (Aug. 1979)	12	Glucose	68	17	0.001	21	38	0.001
C. Coal Bay (Nov. 1979)	8	Glucose	158	54	0.001	22	40	0.001
D. Sadie Cove[a] (Apr. 1979)	13	Glucose	442	74		17	39	
E. Kasitsna Bay (Jan. 1980)	6	Glucose	125	64	0.01	18	41	0.001
F. Kasitsna Bay (Nov. 1979)	8	Glucose	105	38	0.01	18	36	0.01

[a] All observations were made on three or more subsamples except this one in which one observation was made.

[b] $\bar{y}$ = mean values reported in nanograms of substrate taken up × grams (dry weight) of sediment^{-1} × h^{-1}.

[c] Month that the experiment was initiated.

[d] NS = $P > 0.05$.

ments exposed for three different time periods (Fig. 3). In all three studies, there were significant reductions in glucose uptake in sediments exposed to 1 ppt. Concentrations greater than 1 ppt produced no further significant reduction in uptake rates. In the 1- and 3-month studies, measurable reductions were observed at 0.5 ppt, the lowest concentration used in these studies. In the 1-year exposure study, there was a 28% reduction in uptake rates at 0.1 ppt, but this decrease was not statistically significant. A significant reduction in glucose uptake (47%) was also observed in sediments that had been exposed to weathered crude oil for 1 year at a concentration of 1.0 ppt (Table 3). These data suggest that the reduced relative microbial activity that we have observed in this study could also take place in marine sediments that have been contaminated during an actual oil spill.

Because there was an apparent lag in the onset of the glucose uptake rate depression in the Elson Lagoon sediments exposed to crude oil, we determined how long it would take to observe changes in Kasitsna Bay sediments exposed to crude oil. We conducted this study on three marine sediments that were exposed to fresh Cook Inlet crude oil at 50 ppt (Fig. 4). All three of these sediments showed a maximum effect within the first 1.5 days after the initial exposure. In two of the sediments, a maximum effect was observed within the first 12 h. The speed at which this change took place suggests to us that crude oil may have a direct toxic effect on microbial function in the initial stages of exposure. It also serves to sharpen the distinction between the response of an Arctic sediment (Elson Lagoon) and a subarctic sediment (Kasitsna Bay). In the Arctic sediments, no significant depression in uptake rate was observed in a sediment exposed for up to 6 months.

The rapid effect of fresh crude oil on relative microbial activity should be noted by those who

might use this variable to assess the impact of crude oil on subarctic marine sediments. Unless the initial measurements are made within a matter of hours after crude oil becomes incorporated into marine sediments, observations on impacted sediments cannot be used as a control for subsequent observations.

During the Kasitsna Bay study, we were able to follow the effects of fresh crude oil for only 18 months. At the end of this period, the effects of the remaining crude oil were somewhat less than that observed near the beginning of the study. By fitting these data points to the best-fitting power and linear curves for both glucose and glutamic acid uptake, we were able to estimate how long it would take before we would no longer be able to measure an effect on substrate uptake. For glucose uptake, the projected length of effect was 32 and 82 months using the power and the linear curve, respectively. For glutamic acid, the projected length of effect was 20 and 40 months, respectively. Thus, it seems possible that, under similar conditions, one could expect to find measurable effects on microbial function up to 6 years after the initial impact. Considering the relatively high crude oil concentration used, this should represent a maximum impact.

TABLE 2. *Long-term effects of crude oil at 50 ppt on glucose and glutamate uptake and percent respiration in an Elson Lagoon sediment*

Exposure time (months)	Substrate	Uptake rate[a]		Respiration (%)	
		Control	Oil	Control	Oil
6[b]	Glucose	1.6	5.2	23	25
	Glutamate	11	25	45	47
13	Glucose	13.2	6.9	29	23
	Glutamate				
18	Glucose	2.4	0.8	48	71
	Glutamate	7	2	76	90
25	Glucose	172	25	20	38
	Glutamate	1,025	152	48	49
30	Glucose	21	8	28	32
	Glutamate	141	75	50	63

[a] Nanograms × grams (dry weight) of sediment^{-1} × h^{-1}.

[b] Three subsamples were analyzed at this time; all values given are means of those observed values. Three assays were run for each subsample. All other values listed are the mean values observed in three assays on one subsample.

All of the above-mentioned studies were conducted using the one concentration method for determining relative microbial activity (10). By this method, it is impossible to differentiate between competitive inhibition and other mechanisms of inhibition. It is theoretically possible to make this distinction by the use of a multiple concentration method from which uptake kinetics can be calculated (20). These measurements were made using ^{14}C-labeled glutamic acid in sediments that had been exposed to fresh crude oil for 1.5, 5, 11, and 18 months (Table 4). In all cases, there was a significant reduction in the calculated values for the maximum potential uptake rates (V_{max}). If the reduction in uptake rates that we observed was due to competitive inhibition, we should not have observed this change. In addition to this change, we also observed a significant increase in the turnover time in all sediments except those exposed to crude oil for 18 months. We also observed a decrease in the transport constant plus the natural substrate concentration ($K_k + S_n$) values, but this was statistically significant in only one instance. These data suggest that a basic physiological or population change or both have occurred in sediments exposed to fresh crude oil.

During the course of our experiments, we also calculated respiration percentages by using the

TABLE 3. *Effects of weathered crude oil and crude oil-sediment overlays on glucose uptake and respiration percentages in subarctic sediments*

Oil type	Uptake				Respiration (%)			
	Control ($\bar{y}$)[a]	Oil ($\bar{y}$)[a]	% Change[b]	$P <$	Control ($\bar{y}$)[a]	Oil ($\bar{y}$)[a]	% Change[b]	$P <$
A. Fresh vs. "weathered" crude oil								
Fresh, 50 ppt	68	17	−75	0.001	21	38	+81	0.001
Fresh, 1 ppt	68	26	−62	0.01	21	27	+29	0.05
Weathered, 50 ppt	68	25	−63	0.001	21	32	+52	0.01
Weathered, 1 ppt	68	39	−47	0.01	21	20	−5	NS
B. Fresh vs. weathered crude oil overlays								
Fresh	107	37	−65	0.05	23	29	+27	0.05
Weathered	107	121	+13	NS	23	24	+4	NS

[a] $\bar{y}$ = mean value reported in nanograms of glucose taken up × grams (dry weight) of sediment^{-1} × h^{-1}.

[b] Percent change relative to the control.

levels of radioactivity in both the cell and CO_2 fractions. When the sediments were exposed to fresh crude oil at 50 ppt, we always observed a significant increase in the respiration percentages with glucose regardless of the exposure time (Table 1). Similar increases were also observed when labeled glutamic acid or acetate were used; however, these were not always statistically significant, and the changes were not as large as those observed when glucose was used.

To interpret the results of these studies, it is important to keep in mind what is actually being measured. The total uptake rate is the rate at which the labeled substrate is oxidized to $^{14}CO_2$ plus the rate at which the label becomes associated with the cells. Under the experimental conditions used in this study, the label that is in the cell fraction is associated with the macromolecules of the cells (1). Thus, any change in the amount of radioactivity associated with the cells directly reflects changes in biosynthetic mechanisms.

The shift in the respiration percentages could be caused by either a relative increase in the amount of labeled substrate oxidized to CO_2, a decrease in the radioactivity associated with the cells, or a combination of both of these changes. In most cases, there was a reduction in the radioactivity associated with the cells when glucose was used. This is graphically illustrated in one of the concentration experiments (Fig. 5).

When glutamic acid was used as the test substrate, the radioactivity associated with both CO_2 and cells decreased in samples that had been exposed to crude oil for at least 5 months; i.e., there was a decrease in total uptake but little change in the percent respiration (Table 1). These differences suggest that the toxic effects of crude oil on marine microorganisms is not limited to one function. When both the uptake of an organic substrate into the cell and the respiration of this substrate to CO_2 are affected to approximately the same degree, the transport of substrate into the cell has probably been altered. Another explanation might be that crude oil inhibits the growth of organisms that are more efficient in taking up the test substrate, possibly the case with glutamic acid. If CO_2 evolution rates are not affected but there is a large decrease in the amount of radioactivity associated with the cells, it is quite likely that biosynthetic mechanisms are being affected. This condition was observed when glucose was used as the test substrate.

In addition to these measurements, the total adenylate concentrations and direct counts of bacteria in sediments that had been exposed to fresh crude oil at 50 ppt was measured. At both Kasitsna Bay and Elson Lagoon, a statistically significant reduction in total adenylate concentrations in sediments exposed to fresh crude oil

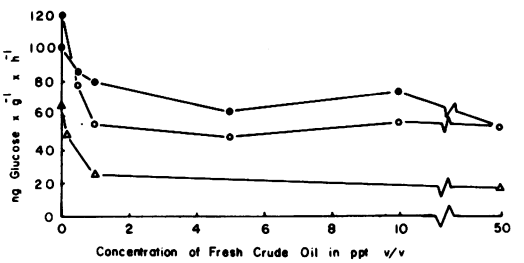

FIG. 3. *Effects of various concentrations of fresh Cook Inlet crude oil on glucose uptake rates in three marine sediments collected in Kasitsna Bay. Symbols for exposure time: ○, 1 month; ●, 3 months; △, 12 months.*

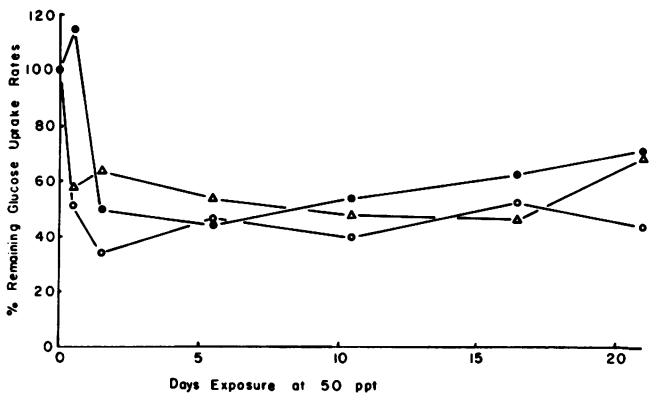

FIG. 4. *Effects of fresh Cook Inlet crude oil at 50 ppt (vol/vol) on glucose uptake rates after different exposure times in three sediments collected in and near Kasitsna Bay. The effect on glucose uptake is expressed as a percent remaining activity relative to the controls (nontreated sediments).*

TABLE 4. *Effects of fresh crude oil (50 ppt) on the kinetics of glutamate uptake in marine sediments*

Determination[a]	Exposure time (months)	Control ($\bar{y}$)	Oil ($\bar{y}$)	% Change	$P <$
V_{max}	1.5	430	160	−63	0.005
	5	1,040	232	−78	0.001
	11	237	89	−62	0.05
	18	1,390	440	−66	0.05
T_t	1.5	85	135	+59	0.05
	5	60	130	+117	0.01
	11	74	205	+131	0.01
	18	23	27	+17	NS
$K_t + S_n$	1.5	20	14	−30	NS
	5	44	18	−60	0.01
	11	33	24	−27	NS
	18	20	13	−35	NS

[a] V_{max}, Maximum potential uptake rate in micrograms of glutamate taken up × grams (dry weight) of sediment^{-1} × h^{-1}; T_t, turnover time in hours; $K_t + S_n$, the transport constant plus the natural substrate concentration.

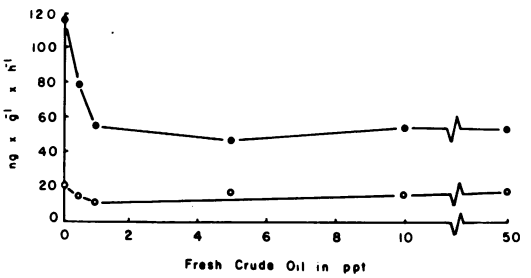

FIG. 5. *Effects of various concentrations of fresh Cook Inlet crude oil on the incorporation of glucose into cell material and respiration to CO_2. The exposure time was 3 months. Symbols: ●, cell; ○, CO_2.*

was observed. In the Kasitsna Bay sediments, there was a 51 and 74% reduction after 1.5 and 5 months of exposure, respectively, and in the Elson Lagoon the observed reduction ranged from 93 to 99% in sediments exposed for 4 to 12 months. We feel that the reduction in total adenylate concentrations in sediments exposed to fresh crude oil reflects a reduction in the infaunal biomass. During sample manipulation, organisms larger than 2 mm were excluded from the subsamples at the time the sediments were analyzed. Thus, this change in total adenylate concentration should reflect a reduction in the biomass of those organisms smaller than 2 mm.

During the Kasitsna Bay long-term crude oil effects study, bacterial concentration determinations using AODC were conducted. After exposure for 1.5 and 5 months, there was a significant reduction (36 and 44%, respectively) in bacterial concentration in sediments that had been exposed to fresh crude oil. Although differences in cell numbers were noted, no difference in average cell size was noted. Therefore the reduction in cell numbers also reflected a reduction in bacterial biomass. After 8 and 18 months of exposure, there was no significant reduction in cell numbers. These data suggest that, during the initial exposure period, at least some of the reduction in substrate uptake rates may be due to a reduction in cell numbers; however, the reductions in substrate uptake observed after longer exposure could not be due to cell reductions. It should be noted that the concentration of bacteria in a given sample does not provide any information about the dynamics of carbon flow through the system. Thus, in the oil-supplemented sediments that had been exposed for 8 months or more, it is possible to have very little change in the bacterial biomass present relative to the controls, yet have a significant difference in the rate at which bacterial biomass is being produced and consumed.

In recent years, it has become increasingly evident that detritus plays an important role as a source of organic nutrients in inshore marine systems. After reviewing the available literature on the subject, Fenchel and Jørgensen (7) estimated that, on the average, approximately 50% of the primary production in marine systems ends up as detritus. They concluded that bacteria play a vital role in making that material available to higher trophic levels by (i) conversion of soluble organic to particulate carbon, (ii) hydrolyzation of recalcitrant organic compounds such as cellulose and chitin, (iii) enriching the detritus with organic nitrogen and phosphate. For the bacterial biomass to become available to the system, the bacteria must be consumed directly or digested from detritus particles while

passing through the guts of higher organisms. Our data suggest that the production of bacterial biomass (i.e., biosynthesis) in marine sediments can be reduced in the presence of crude oil at concentrations which could occur during an actual oil spill. From what is known about nutrient cycling through the benthic detrital food chain, we would predict that crude oil can reduce secondary productivity in Arctic and subarctic marine sediments.

It is quite likely that crude oil may also affect secondary productivity by killing or injuring those organisms that are responsible for the initial transfer of bacterial biomass to biomass at higher trophic levels. During the course of our study, we observed no burrowing activity in sediments that had been exposed to crude oil for up to 18 months, whereas in the control sediments, the sediment was constantly being turned over by the resident infaunal population. The reduction in total adenylates observed in oiled sediments may also reflect a reduction in the eucaryotic bactivorus population which would normally transfer carbon to higher tropic levels. There are a number of reports in the literature suggesting that benthic organisms, which could consume bacteria as part of their diet, are adversely affected by the presence of crude oil (5, 9, 17). Although the disruption of this step in the detrital food chain was not documented in this study, it is another way in which the secondary productivity of the system might be affected by the presence of crude oil in marine sediments. Unfortunately, there have been no cases, of which we are aware, in which an attempt has been made to measure the impact of crude oil or petroleum on secondary productivity in impacted marine sediments.

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