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Field Observations on the Acute Effect of Crude Oil on Glucose and Glutamate Uptake in Samples Collected from Arctic and Subarctic Waters†

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The acute effects of crude oil on glucose uptake rates by marine microorganisms were studied in 215 water and 162 sediment samples collected from both arctic and subarctic marine waters. The mean percentage reduction of glucose uptake rates ranged from 37 to 58 in the water samples exposed to crude oil and from 14 to 36 in the sediment samples. Substrate uptake kinetic studies indicated that the observed reductions by microbial populations exposed to crude oil were caused by metabolic inhibition. The effect of crude oil was less in sediments than in the water samples, with the difference being significant at the $P < 0.0002$ level. With the exception of one sediment study, all of the differences observed in the uptake rates between treated and nontreated samples were statistically significant. A high degree of variability was observed in the degree which glucose and glutamate uptake rates were altered in water samples exposed to crude oil. In some cases, uptake rates were greater in the samples exposed to crude oil. Data on samples collected in Cook Inlet suggested that areas where pelagic microorganisms are most probably chronically exposed to crude oil are also the areas where the effects of crude oil on glucose uptake are the lowest. Two studies indicated that after pelagic populations are exposed to crude oil for several days, the heterotrophic population adjusts to the presence of crude oil.

There have been a number of studies (2-4, 8, 13) addressing the question: What effects do crude oil, refined petroleum products, or pure hydrocarbons have on marine microbial populations? Several of these studies were concerned with the effects of crude oil on microbial growth rates. For the most part, these studies have shown that crude oil has very little effect on growth; however, reduced growth rates have been shown in populations exposed to refined petroleum products (14) or aromatic hydrocarbons (3, 4). In a study of the effects of crude oil on cell numbers in seawater taken from Yaquina Bay, Ore., no significant changes in plate counts were observed in water samples exposed to Prudhoe Bay crude oil for periods of up to 40 days (R. P. Griffiths, unpublished data). Atlas et al. (2) reported that Prudhoe Bay water exposed to crude oil showed an increase in cell numbers relative to the control after 42 days. Although no significant change in species diversity was observed in this experiment, a more recent study by Atlas (personal communication) using numerical taxonomic techniques has shown shifts in the composition of bacterial populations in arctic marine waters exposed to Prudhoe crude oil for several months.

Hodson et al. (8) reported that crude oil-aqueous solutions inhibited the uptake and mineralization of ^{14}C-labeled glucose by pelagic microorganisms. These observations were made in seawater samples contained in large plastic bags during the course of the CEPEX (Controlled Ecosystem Pollution Experiment) project (10). In a more recent study, Alexander and Schwarz (1) reported finding little effect of two crude oils on [^{14}C]glucose uptake and mineralization in 15 sediment and 13 water samples collected in Galveston Bay and off the Louisiana coast. The present study was conducted to determine the short-term effects of two Alaskan crude oils on sediment and water samples collected in subarctic and arctic marine systems.

MATERIALS AND METHODS

Both the sampling procedures and the methods used to determine relative microbial activity and mineralization rates have been described previously (5, 6). The uptake rates reported here were calculated from

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the amount of label remaining in the cells after acidification plus that trapped as $^{14}CO_2$. The sediment samples were diluted 1,000 times (vol/vol) with sterile 30% artificial seawater which was cooled to 5°C. Ten milliliters of the water or sediment suspension was added to a chilled 50-ml serum bottle. Ten microliters of crude oil was added to the surface of the sample. The bottles were then sealed and incubated without shaking in the dark at a temperature that was within 1°C of the in situ temperature. Incubation periods ranged from 1 to 12 h. Crude oil from Prudhoe Bay, Alaska, was used in the Beaufort Sea studies, and oil from Cook Inlet, Alaska, was used in all other cases.

Since crude oil interferes with the cell radioactivity assay, 5 ml of the sample was removed at the end of the experiment with a 5-ml syringe fitted with a cannula. Before the sample was removed, the syringe was filled with air, and the plunger was steadily pushed downward so that the cannula was purged with air as it was passed through the surface oil slick to prevent oil from getting into the cannula. Five milliliters of the sample was then drawn into the syringe and dispensed onto a membrane filter for further processing.

The glucose used in these experiments was D-[*U*-^{14}C]glucose (Amersham/Searle) with a specific activity of 304 to 328 mCi/mmol. The final substrate concentration in the reaction vessels ranged from 6 to 7 μg/liter. L-[*U*-^{14}C]glutamic acid (Amersham/Searle) was used in the April 1978 Cook Inlet study. In sediment samples, glutamate with a specific activity of 10 mCi/mmol and a final concentration of 73 μg/liter was used. In water samples, glutamate with a specific activity of 278 mCi/mmol and a final concentration of 2.7 μg/liter was used. When the multiconcentration method (15) was used to determine uptake kinetics, D-[*U*-^{14}C]glucose was used at concentrations ranging from 1.5 to 12 μg/liter. In all single-substrate concentration experiments, triplicate subsamples were analyzed; in multiconcentration experiments, duplicate subsamples at four concentrations were analyzed.

Two methods were used for time course studies. In the Elson Lagoon water sample study, 2 liters of seawater was added to a sterile 4-liter glass container. Two containers were used, one nontreated control and one to which 1 ml of Prudhoe Bay crude oil was added. These containers were fitted with sterile glass siphon tubes through which subsamples were collected. The containers were incubated without mixing in the dark at 4°C for 1.5 to 8 h. At various time intervals, subsamples were removed for analysis. These subsamples were then split into two portions; one was used to assay acetate uptake and respiration (mineralization), and the other was used for glutamate uptake measurements. Uptake kinetics were determined by procedures described previously (6). The same glutamate was used in this experiment as that described above. The acetate used was [*U*-^{14}C]acetic acid, sodium salt (Amersham/Searle), with a specific activity of 59 mCi/mmol and a final concentration range of 3 to 26 μg/liter.

When Kasitsna Bay water samples were analyzed, another procedure was used. Seawater subsamples of 500 ml were added to each of two 1-liter sterile glass bottles. To these bottles was added enough filter-sterilized sodium glutamate to give a final concentration of 50 mM. At the start of the experiment, 0.5 ml of Cook Inlet crude oil was added to one of the bottles. At various time intervals, triplicate 10-ml subsamples were removed from each bottle with a syringe and used to assay glucose uptake and respiration. The samples were incubated at 8°C in the dark without shaking for 1 to 3 h.

Differences in mean values were analyzed by Student's *t* test. A critical value of $P < 0.05$ was used to define the significant difference.

RESULTS AND DISCUSSION

We studied the effects of Alaskan crude oil on 215 water and 162 sediment samples collected from three very different regions along the Alaskan coast, all of which could be affected by crude oil production or transportation (Fig. 1). One goal of this study was to determine whether the presence of crude oil would alter relative microbial activity and percentage respiration in these diverse marine environments. We found that in all field studies, there was a statistically significant difference between the oil-treated and non-oil-treated samples (Table 1). The range in the mean percentage reduction values observed for glucose uptake rates was from 37 to 58. The statistical significance of these differences ranged from $P < 0.035$ to $P < 0.00001$.

In seven of eight field studies where sediment samples were analyzed, there was also a statistically significant difference between glucose uptake rates in treated and nontreated sediments (Table 1). The mean percentage reduction values observed in the sediment samples ranged from 14 to 36. The differences in the mean values observed in different regions were not significant. Even though both pelagic and benthic microorganisms were affected by the presence of

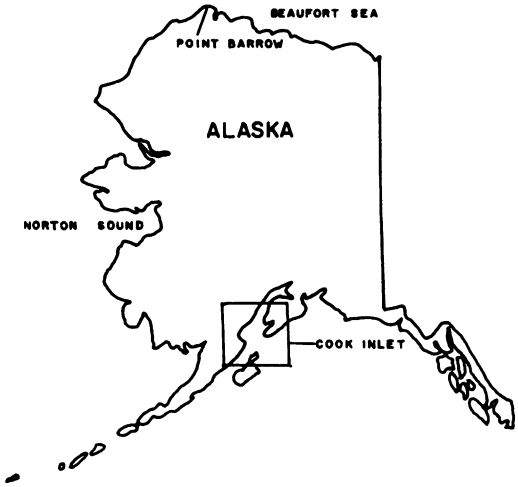

FIG. 1. *Location of sampled region relative to the state of Alaska.*

TABLE 1. *Percentage reduction of glucose uptake rates in microbial populations exposed to crude oil*

Sample	Mean $\bar{y}$	Standard deviation	No. of samples	Range	P^a
Water					
Beaufort Sea, February 1978	52	20	40	−8–88	<0.0001
Cook Inlet, April, 1978	45	30	32	−43–86	<0.01
Cook Inlet, May 1979	58	20	47	−22–92	<0.0005
Norton Sound, July 1979	57	15	60	18–82	<0.00001
Kasitsna Bay, February 1979	37	13	7	22–83	<0.012
Kasitsna Bay, April 1979	58	16	6	36–72	<0.035
Kasitsna Bay, July 1979	56	11	23	0–72	<0.0001
Sediment					
Beaufort Sea, September 1977	35[b]	18	20	0–65	<0.002
Beaufort Sea, September 1978	32	22	34	0–68	<0.001
Cook Inlet, April 1978	14	32	14	−73–35	NS
Cook Inlet, May 1979	29	42	14	−52–78	<0.048
Norton Sound, July 1979	36	22	34	1–73	<0.0001
Kasitsna Bay, February 1979	25	14	12	0–49	<0.01
Kasitsna Bay, April 1979	16	13	11	−2–42	<0.01
Kasitsna Bay, July 1979	30	12	23	0–72	<0.0001

[a] Level of statistical significance between treated and nontreated samples. NS, Not statistically different at the $P < 0.05$ level.

[b] CO_2 data only.

crude oil, the benthic microorganisms were affected to a lesser degree. The statistical significance of that difference was at the $P < 0.0002$ level. The reason for this difference is not known; perhaps the benthic microorganisms are more consistently exposed to biogenic hydrocarbons.

The field studies were conducted by using 10 μl of fresh crude oil in a 10-ml sample. We compared the effects of this treatment on glucose uptake rates with that observed when 100 μl was added to the same sample volume. There was no statistically significant difference between the effects observed with these two treatments (Griffiths, unpublished data). It was therefore assumed that the amount of crude oil added to the samples in the field studies was enough to saturate the system with dissolved hydrocarbons.

All of the above-mentioned studies were conducted with labeled glucose. We wanted to determine whether a similar crude oil effect would be observed if an amino acid was used as a substrate in determining relative microbial activity. During the 1978 Cook Inlet cruise, an experiment was conducted on 35 water and 7 sediment samples, using labeled glutamic acid. The mean percentage reduction observed was 33 and 18 for water and sediment samples, respectively. The difference observed in the sediment samples was not statistically significant. When the water samples were analyzed in the same way, however, the difference between oiled and nonoiled samples was significant at the $P < 0.0003$ level. A detailed listing of the data (Table 2) shows that when the replicates of the same sample were analyzed individually, 20 of the 35 samples tested showed a significant reduction in glutamic acid uptake rates in oiled water samples ($P < 0.05$). If the confidence limit is changed to $P < 0.10$, 27 of the 35 samples showed a significant reduction. It can thus be said that at least in pelagic microbial communities, the effect of crude oil on heterotrophic rates is not limited to glucose uptake.

Since the single-concentration method (5) was used to measure changes in uptake and respiration rates in populations exposed to crude oil, it can be argued that the observed difference might have been caused by some component or components of the crude oil which were competing for the same transport mechanisms being used by the test substrates. To determine whether this was the case, we elected to use the Wright and Hobbie (15) technique for measuring uptake kinetics. By using this technique, the maximal potential uptake rate (V_{max}), the turnover time required to utilize all of the naturally occurring substrate by the microbial population (T_t), and transport constant plus the natural substrate concentration ($K_t + S_n$) can be calculated. If some component of crude oil is being transported into the cells via the same mechanism as glucose, V_{max} should not change, but T_t and $K_t + S_n$ should increase. A study on the effects of crude oil on the kinetics of glucose uptake was conducted on six water samples collected in the Beaufort Sea. Mean V_{max} decreased from 14.6 in the nontreated samples to 3.7 μg/liter per h in the treated samples. Mean T_t increased from 177 to 492 h, and $K_t + S_n$ remained unchanged. The differences observed in both V_{max} and T_t were statistically significant. It thus appears that

TABLE 2. *Effects of fresh crude oil at 0.1% (vol/vol) on labeled glutamic acid uptake in water samples collected in Cook Inlet during the April 1978 cruise*

Sample no.	Uptake (ng/liter per h)				% Change	P[a]
	Nonoiled		Oiled			
	Mean[b]	Standard deviation	Mean[b]	Standard deviation		
648	1.7	0.1	1.5	0.06	−12	0.07
649	10.0	0.1	3.3	0.4	−67	0.003
651	93	35	44	13	−53	0.09
652	33	2	16	0.5	−52	0.0008
653	2.5	0.7	1.3	0.1	−48	0.05
654	4.3	0.3	2.6	0.1	−40	0.002
655	7.2	0.9	4.3	1.4	−40	0.01
656	25	2	18	1.2	−26	0.008
657	6.8	1.1	10	0.3	+47	0.14
658	15	2	11	2	−25	0.15
659	10	2	5.6	1.8	−44	0.07
660	72	4	24	7	−67	0.002
661	63	7	28	12	−56	0.003
662	9.9	0.2	5.8	0.2	−41	0.0003
663	18	2	6.5	2	−64	0.006
664	17	4	13	0.8	−24	0.08
665	13	1	10	1	−23	0.07
666	10	2	7	0.6	−30	0.04
667	20	2	11	4	−45	0.03
668	34	0.9	17	4	−50	0.005
669	103	34	41	12	−60	0.04
670	58	3	51	5	−12	0.01
671	61	9	34	4	−44	0.01
672	69	1	41	3	−41	0.0007
673	81	4	62	8	−23	0.04
674	95	5	83	2	−13	0.02
675	22	7	22	5	0	0.64
676	10	0.5	10	2	0	0.57
677	15	1	6	2	−60	0.003
678	21	0.6	24	8	+14	0.37
679	15	2	11	0.5	−27	0.21
680	10	3	4	0.6	−50	0.02
681	2.5	0.7	23	0.3	−8	0.57
682	4.6	0.2	4.4	0.1	−4	0.14
683	30	5	14	0.5	−53	0.06

[a] As described in Table 1.
[b] Of three replicates.

crude oil was not acting as a competitive inhibitor.

During the course of our studies, we also measured the percentage respiration. We did observe differences in the percentage respiration between treated and nontreated samples; however, these differences were not consistent, and they were not statistically significant at the $P \leq 0.05$ level in most cases. The mean values for treated samples were usually slightly higher than those observed in nontreated samples. These data suggest that the microbial function most affected was substrate transport. If either biosynthetic or respiratory functions were consistently affected, there would have been a significant change in the percentage respiration values in treated samples.

Even though the glucose and glutamate uptake rates were generally reduced in the presence of crude oil, a wide range of effects was observed; in some cases, the uptake rate was actually higher in the treated sample (Tables 1 and 2). The degree to which water samples were affected by the presence of crude oil was analyzed in terms of sample location. During the 1978 Cook Inlet cruise, the patterns of effect in water samples suggested that populations which most probably had been exposed to chronic petroleum perturbation may not have been as greatly affected by crude oil as those that had not been exposed (Fig. 2). A series of consecutive water samples was collected on a transect starting near Augustine Island and ending near Homer, Alaska. The water samples collected near

the center of the shipping lane (circled station in Fig. 2) showed less effect than those collected at either end of the transect. When the differences in the effect of crude oil on glutamate uptake on water samples collected at the three stations in and closest to the shipping channel were compared with the effects observed at the outer stations, the difference was significant at the $P = 0.05$ level. The center samples were located in a region of little net water flow which is also in the center of the shipping lane. One sample tested in this region showed higher uptake and respiration rates in the subsample exposed to crude oil than that observed in the control (the sample with the negative percentage reduction in Fig. 2). It is assumed that petroleum products are constantly being introduced into these waters as the result of shipping.

Water samples were also taken at two locations just north of Augustine Island. The one to the east was taken in Oil Bay, which is so named because of a natural oil seep in that region (E. Wood, personal communication). The one to the west was taken in a similar bay in which no seep has been reported. The reduction in glutamate uptake in the water sample taken from Oil Bay was only 12%, compared with a reduction of 67% observed at the other location (the reduction in the glucose uptake in the same samples was 0 and 61%, respectively).

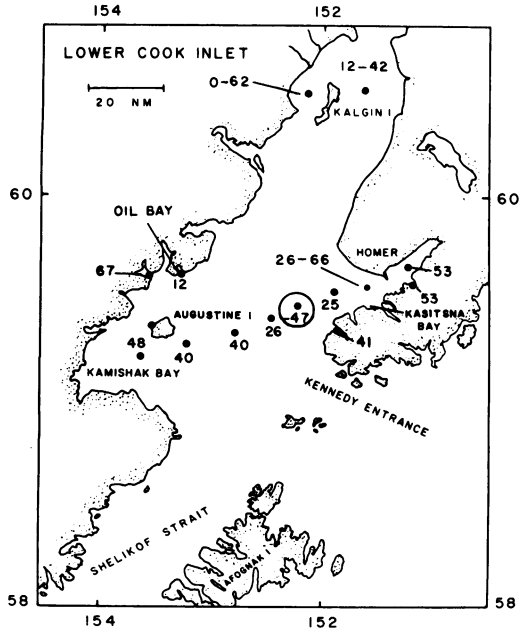

FIG. 2. *Percentage reduction in gluatamate uptake rates in water samples collected in Cook Inlet which have been exposed to crude oil. The circled station indicates the sample in which an increased uptake rate was observed in the oil-treated water.*

A series of observations was also made in water samples collected at three sample locations, one near Homer and two to the north near Kalgin Island. Figure 2 shows the range of values observed at these locations. The percentage reduction values observed in water samples collected at the northern stations were significantly lower than those observed in the station near Homer. The water mass at the sample site near Homer consists primarily of open ocean water which should have had little prior exposure to petroleum products. The northern sample sites are located near oil wells in the inlet and also near an oil refinery with associated shipping facilities. Elevated levels of petroleum hydrocarbons have been observed in the waters to the north of these sample sites (R. Feely, personal communication). Roubal and Atlas (12) reported high concentrations of hydrocarbon-utilizing bacteria in the water samples collected at the two northern sample sites. There have been numerous reports in the literature linking elevated concentrations of hydrocarbon-utilizing bacteria with petroleum contamination in the marine environment (7, 9).

These observations suggest that the natural microflora can adjust to the presence of petroleum hydrocarbons. To test this hypothesis, we conducted two experiments using two crude oils and water samples collected from two sources. In the first study, we exposed water collected from Elson Lagoon near Point Barrow in the Beaufort Sea to Prudhoe Bay fresh crude oil and monitored the relative microbial activity, using labeled glutamic acid and acetate, in water samples which were exposed to oil for various periods of time (Fig. 3). After 1.5 days of exposure, there was very little difference between the V_{max} observed in oiled versus nonoiled waters. At 3.5 days, the "bottle effect" was observed with a significant increase in relative microbial activity, reflecting what we presume to be an increase in cell numbers. When either glutamate or acetate was used to measure relative microbial activity, the uptake rates were significantly lower in the oiled water samples which had been exposed to crude oil for 3.5 days. After an incubation period of 9.5 days, the relative microbial activity as measured by either glutamate or acetate was significantly higher in the oiled samples than in the nonoiled controls.

The second study was conducted under much different conditions, but the results were essentially the same. In this experiment, a water sample was collected from Kasitsna Bay (Cook Inlet) and exposed to Cook Inlet crude oil. In

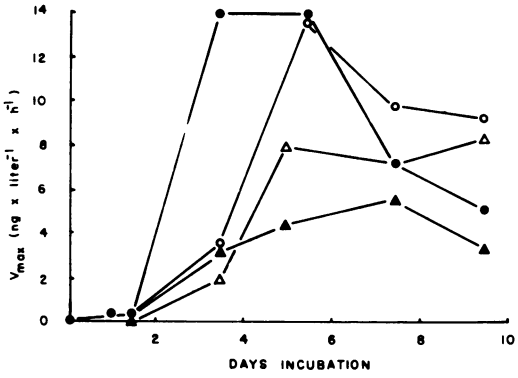

FIG. 3. *Effects of fresh Prudhoe Bay crude oil on relative microbial activity as measured with glutamic acid or acetate uptake. Symbols: ○, Glutamate uptake rates in oiled sample; ●, glutamate uptake in nonoiled sample; △, acetate uptake in oiled sample; ▲, acetate uptake in nonoiled sample.*

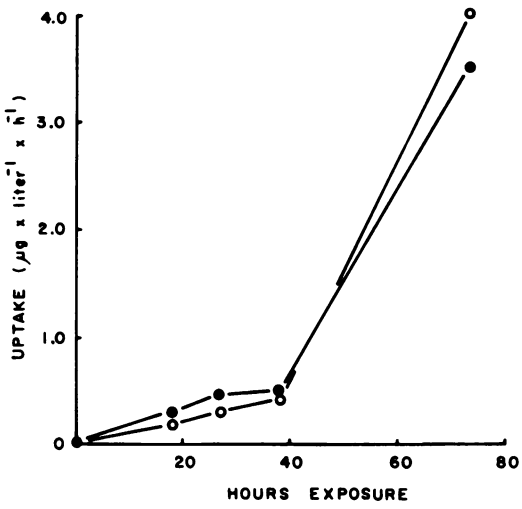

FIG. 4. *Effects of fresh Cook Inlet crude oil on relative microbial activity as measured by glucose uptake. Symbols: ○, Oiled sample; ●, nonoiled sample.*

addition, unlabeled glutamic acid at a concentration of 50 mM was added to the water sample. The relative microbial activity was monitored with one concentration of labeled glucose. During the first 27 h of the experiment, the relative microbial activity in the oiled sample was significantly lower than that observed in the control (Fig. 4). After 38 h, the activity levels were essentially the same in both samples; after 74 h, the relative microbial activity in the oiled sample was significantly higher than in the nonoiled control. Although determinations of bacterial numbers were not made during these experiments, the uptake data strongly suggested that after extended exposure, the growth of pelagic heterotrophic microorganisms was not adversely affected by the presence of these two crude oils.

Both this study and that reported by Hodson et al. (8) indicate that there is a significant reduction in the heterotrophic uptake rates in pelagic microbial populations when they are first exposed to crude or refined oils. This effect was observed when either glucose or glutamic acid was used as the test substrate, indicating that this phenomenon is not restricted to glucose (the substrate used by Hodson et al. [8]). The phenomenon is caused by a number of crude oil types and crude oil products and affects a large cross section of natural marine microbial populations, although pelagic populations appear to be affected to a greater extent than benthic populations.

The results of the study of Hodson et al. (8) and this study differ from those reported by Alexander and Schwarz (1) in a similar study. When using south Louisiana and Kuwait crude oils at concentrations equivalent to those used in this study, they observed reductions in glucose uptake and mineralization rates in only one sediment and two water samples out of 15 and 13 samples, respectively. While comparing their results with those of Hodson et al. (8), they suggested that the differences in the results were most probably due to differences in the oils used in the two studies. The results of our study suggest another explanation. The samples that Alexander and Schwarz (1) used were collected in Galveston Bay and from two locations off the Louisiana coast near the Mississippi River. The Buccaneer oil field is located offshore from Galveston Bay, and there is active transport of petroleum products along the Mississippi River and within Galveston Bay. Thus, it is very likely that the sediments and waters of the region studied by Alexander and Schwarz (1) have been chronically exposed to crude oil, petroleum products, or both. If this is the case, the populations they studied may have adjusted to the presence of crude oil before their experiments. Most areas that we have studied have had little previous exposure to petroleum hydrocarbons. The same may also be true of the samples tested by Hodson et al. (8), which were collected off Vancouver Island in British Columbia.

Our results suggest that substrate uptake and growth rates in marine pelagic microbial populations may initially be inhibited by the presence of crude oil but that this population will adjust to this perturbation within a matter of days. Since the experimental conditions were designed to saturate the water with soluble hydrocarbons, the effects observed in the study probably rep-

resent the greatest effect possible. Under actual spill conditions, the water directly adjacent to the surface slick should contain approximately the same concentration of dissolved hydrocarbons as that used in this study. The extent of the dissolved hydrocarbon effect on the pelagic microbial community would be dependent on the extent of the oil spill and the rate at which hydrocarbons from the slick became incorporated into the water column.

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