Radiolabelling of Sedimentary Organic Matter with $^{14}$C-Formaldehyde: Preliminary Evaluation of a New Technique for Use in Deposit-Feeding Studies*

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ABSTRACT: Organic matter in marine sediments can be sufficiently labelled for ingestion experiments with deposit-feeders by allowing $^{14}$C-formaldehyde to bond chemically to it. Microbes, which otherwise would account for much of the $^{14}$C-formaldehyde uptake, are reversibly inhibited with 30% NaCl. When incubated in 30% NaCl, at least 95% of the $^{14}$C uptake appears to be due to chemical bonding with sedimentary organics. There is a positive relationship between $^{14}$C-formaldehyde uptake and organic content of sediment.

INTRODUCTION

The goal of this study was to develop a method of radiolabelling sedimentary organic matter for use in feeding studies on deposit-feeding animals. Two approaches have been used previously for such studies: (1) Chemical/gravimetric analyses of natural material (Newell, 1965; Hargrave, 1970; Lopez et al., 1977); (2) radiolabelled ‘synthetic’ detritus (Kofoed, 1975a; Tenore, 1977; Cammen, 1980). One disadvantage of the first approach is its lack of precision and sensitivity in measuring small changes, a typical problem in estimating assimilation of organic debris by animals. For example, it is usually impossible to distinguish 5% assimilation from 0% assimilation. Chemical/gravimetric analyses are not easily applied to study ingestion selectivity of organic matter, a subject of considerable interest in deposit-feeding research (Self and Jumars, 1978; Taghon et al., 1978; Lopez and Kofoed, 1980; Lopez and Cheng, 1982). The use of radiolabelled detritus prepared in the laboratory allows greater sensitivity for measuring small changes (e.g. Kofoed, 1975a, b), but studies using such material always suffer in extrapolation to the natural world. In this study we have developed methods which allow the use of radiotracers to study the ingestion of natural sedimentary organic matter by deposit-feeders. Similar methods have been described by Banks and Wolfinbarger (1981).

For ingestion experiments, 2 conditions must be met by the radiolabelling procedure: The label must be associated with sedimentary organic matter, rather than be adsorbed to surfaces or taken up by microbes, and the label should not migrate during the time course of the feeding experiment. It is important to note that the conditions for ingestion experiments are less rigorous than those to study assimilation, which requires the added condition of knowing the distribution of radioisotope within the labelled substrate (Lampert, 1977; Banks and Wolfinbarger, 1981).

MATERIALS AND METHODS

$^{14}$C-formaldehyde was chosen as labelling agent, because formaldehyde reacts with a wide variety of functional groups, perhaps the most common reaction being the formation of a cross-link of functional groups with a methylene bridge (Pearse, 1968, pp. 70–73). Because preliminary experiments with phenol- and heat-killed sediments suggested that microbial activity could account for most of the $^{14}$C-formaldehyde uptake, this study was divided into 2 aspects:

* Contribution No. 318 from the Marine Sciences Research Center, State University of New York at Stony Brook

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0171-8630/81/0008/0283/$ 02.00
Development of a technique that inhibits microbial uptake of $^{14}$C-formaldehyde without interfering with physical/chemical uptake; (2) determining the relationship between physical/chemical uptake of $^{14}$C-formaldehyde and sedimentary organic matter.

**Microbial Inhibition with 30 % NaCl**

Distinguishing microbial from physical/chemical processes is a common problem in biogeochemical studies, and interpretation of results can hinge on the type of microbial control used (Brock, 1978). The problem is obvious in this study; clearly, formaldehyde fixation is not suitable. Furthermore, because the purpose of this study is to develop methods for use in feeding experiments, the labelling method should not leave toxic residues in the sediment. Therefore, we have investigated the efficacy of a concentrated solute, 30 % NaCl, as an inhibitor of microbial activity (Brock, 1978).

**Experiment 1: Effect of 30 % NaCl on $^{14}$C-mixed Amino Acid Uptake by Marine Sediment**

This experiment was conducted to determine whether 30 % NaCl affects microbial activity associated with a marine sediment, as measured by incorporation of $^{14}$C-mixed amino acids (U-14C protein hydrolysate, 57 mCi mAtom$^{-1}$, Amersham) from solution. A sediment suspension was prepared from the light fraction of an intertidal muddy sediment. Sediment suspensions (5 ml, ~ 65 mg dry weight) were pre-incubated for 1.75 h in either seawater, 30 % NaCl—both of which were filtered (0.45 µm) before use—or a HgCl$_2$ solution (90 µg ml$^{-1}$ seawater). Samples were centrifuged and resuspended in one of the solutions and allowed to sit for 15 min. $^{14}$C-mixed amino acids (0.5 µCi) were then added to each sample. After 1 h incubation at 25 °C, subsamples were filtered onto Nuclepore filters (1 µm pore size, 25 mm diameter). Filters were placed in glass liquid scintillation vials and solubilized in 0.5 ml tissue solubilizer (NCS, Amersham) for 16 h at 45 °C. After adding 10 ml organic scintillant (OCS, Amersham), samples were counted on a Nuclear-Chicago Mark II liquid scintillation counter, and counts were corrected to dpm using the external standard ratio method with quench standards prepared with NCS-sediment quenching agent.

The results of Experiment 1 suggested that microbial inhibition by 30 % NaCl was at least partly reversible. In Experiment 2, we investigated this apparent reversibility of inhibition. Sediment suspensions, prepared as in the previous experiment, were pre-incubated in either seawater or 30 % NaCl, then allowed to recover for 18 or 48 h before incubating with $^{14}$C-mixed amino acids. Incubation and sampling were performed as in the previous experiment.

**Experiment 2: Effect of Varying Recovery Periods Following 30 % NaCl Treatment on Microbial Uptake of $^{14}$C-mixed Amino Acids**

An independent method of determining the effect of 30 % NaCl on microbial activity, in this case on $^{14}$C-formaldehyde uptake, is to compare uptake in 30 % NaCl and in seawater over a wide range of incubation temperatures (Brock, 1978). If 30 % NaCl is an effective inhibitor of microbial activity, the seawater sample should exhibit higher uptake (microbial + physical/chemical) than the 30 % NaCl sample (physical/chemical only) when incubated at non-lethal temperatures. At temperatures that inactivate or kill microbes, seawater and 30 % NaCl samples should display the same activity. Samples of a sediment suspension were centrifuged and resuspended in 10 ml seawater or 30 % NaCl, and then pre-incubated at experimental temperatures (2, 8, 23, 34, 60, and 75 °C) for 1 h before addition of $^{14}$C-formaldehyde. The stock solution of $^{14}$C-formaldehyde was prepared by diluting 500 µCi (10 mCi mmol$^{-1}$, 1–3 % aqueous solution, Amersham) in 100 ml distilled water (stock concentration 5 x 10$^{-4}$ M). After preincubation, 0.2 ml (1 µCi) $^{14}$C-formaldehyde was added to each sample, which was then stoppered, shaken and incubated at the experimental temperatures for 44 h. After incubation, samples were centrifuged and resuspended in seawater or 30 % NaCl, according to treatment, to remove unincorporated $^{14}$C-formaldehyde. This step was important because $^{14}$C-formaldehyde binds with filters when the suspension is filtered during sampling. Subsamples were then filtered onto glass fiber filters (Whatman, GF/A, 25 mm), rinsed with 5 ml seawater or 30 % NaCl, and prepared for liquid scintillation counting as described above.

**Experiment 3: Effect of Incubation Temperature and 30 % NaCl on $^{14}$C-formaldehyde Uptake**

An independent method of determining the effect of 30 % NaCl on microbial activity, in this case on $^{14}$C-formaldehyde uptake, is to compare uptake in 30 % NaCl and in seawater over a wide range of incubation temperatures (Brock, 1978). If 30 % NaCl is an effective inhibitor of microbial activity, the seawater sample should exhibit higher uptake (microbial + physical/chemical) than the 30 % NaCl sample (physical/chemical only) when incubated at non-lethal temperatures. At temperatures that inactivate or kill microbes, seawater and 30 % NaCl samples should display the same activity. Samples of a sediment suspension were centrifuged and resuspended in 10 ml seawater or 30 % NaCl, and then pre-incubated at experimental temperatures (2, 8, 23, 34, 60, and 75 °C) for 1 h before addition of $^{14}$C-formaldehyde. The stock solution of $^{14}$C-formaldehyde was prepared by diluting 500 µCi (10 mCi mmol$^{-1}$, 1–3 % aqueous solution, Amersham) in 100 ml distilled water (stock concentration 5 x 10$^{-4}$ M). After preincubation, 0.2 ml (1 µCi) $^{14}$C-formaldehyde was added to each sample, which was then stoppered, shaken and incubated at the experimental temperatures for 44 h. After incubation, samples were centrifuged and resuspended in seawater or 30 % NaCl, according to treatment, to remove unincorporated $^{14}$C-formaldehyde. This step was important because $^{14}$C-formaldehyde binds with filters when the suspension is filtered during sampling. Subsamples were then filtered onto glass fiber filters (Whatman, GF/A, 25 mm), rinsed with 5 ml seawater or 30 % NaCl, and prepared for liquid scintillation counting as described above.

**Relationship Between Physical/Chemical Uptake of $^{14}$C-formaldehyde and Sedimentary Organic Matter**

**Experiment 4: Effect of Sedimentary Organic Matter on $^{14}$C-formaldehyde**

Seven sediments ranging from highly organic mud to clean sand were rinsed in distilled water and dried
Table 1: Experiment 1: Effect of 30% NaCl on $^{14}$C-mixed amino acid uptake by marine sediment

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pretreatment (1.75 h)</th>
<th>Treatment (1 h)</th>
<th>dpm ($\bar{x} \pm$ s.d., n = 2)</th>
<th>% of I</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>seawater</td>
<td>seawater</td>
<td>168,192 ($\pm$ 27,370)</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>HgCl₂</td>
<td>HgCl₂</td>
<td>2,145 ($\pm$ 11)</td>
<td>1.3</td>
</tr>
<tr>
<td>III</td>
<td>HgCl₂</td>
<td>seawater</td>
<td>2,469 ($\pm$ 15)</td>
<td>1.5</td>
</tr>
<tr>
<td>IV</td>
<td>30% NaCl</td>
<td>HgCl₂</td>
<td>1,647 ($\pm$ 199)</td>
<td>1.0</td>
</tr>
<tr>
<td>V</td>
<td>30% NaCl</td>
<td>30% NaCl</td>
<td>3,125 ($\pm$ 383)</td>
<td>1.9</td>
</tr>
<tr>
<td>VI</td>
<td>30% NaCl</td>
<td>seawater</td>
<td>60,496 ($\pm$ 3,618)</td>
<td>36.0</td>
</tr>
</tbody>
</table>

at 105 °C. Portions of each sediment were then ashed at 500 °C for 2 d. Dried and ashed sediments (~ 0.3 cm³) were then placed in 10 ml 30% NaCl and allowed to rehydrate for 2 d. $^{14}$C-formaldehyde (0.5 μCi) was added, and samples were incubated at 25 °C for 45 h. Samples were then centrifuged and resuspended in 30% NaCl. Muddy sediments (1, 2, 3, 4) were subsampled by filtering portions onto preweighed Nuclepore filters. Sandy sediments were sampled with small, preweighed aluminum planchets (Rublee and Dornseif, 1978). After drying and weighing, subsamples were placed in scintillation vials, dampened with water, and then solubilized with tissue solubilizer and prepared for counting. Organic content of sediments was estimated by weight loss upon ashing.

Experiment 5: Loss of $^{14}$C from Sediment Labelled with $^{14}$C-formaldehyde

A fresh sediment suspension in 30% NaCl was labelled for 44 h with $^{14}$C-formaldehyde, rinsed and transferred to seawater. Subsamples were then taken, beginning 5 h after transferring. Subsampling continued for 4 d. Sampling and counting procedures were similar to those used in Experiment 4.

RESULTS

Experiment 1

Samples pre-incubated and incubated in seawater exhibited highest $^{14}$C-amino acid uptake of any of the treatments (Table 1). Samples subjected to HgCl₂ during pre-incubation or incubation displayed only 1 to 1.5% of the activity of the seawater control. Incubation

Table 2: Experiment 2: Effect of varying recovery periods following 30% NaCl incubation on microbial uptake of $^{14}$C-mixed amino acids

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pretreatment 1.5 h</th>
<th>Treatment</th>
<th>dpm ($\bar{x} \pm$ s.d., n = 2)</th>
<th>% of I</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>seawater</td>
<td>seawater</td>
<td>292,923 ($\pm$ 10,330)</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>30% NaCl</td>
<td>30% NaCl</td>
<td>11,118 ($\pm$ 2,085)</td>
<td>3.8</td>
</tr>
<tr>
<td>III</td>
<td>30% NaCl</td>
<td>seawater</td>
<td>322,104 ($\pm$ 9,105)</td>
<td>110</td>
</tr>
<tr>
<td>IV</td>
<td>seawater</td>
<td>seawater</td>
<td>175,757 ($\pm$ 10,607)</td>
<td>100</td>
</tr>
<tr>
<td>V</td>
<td>30% NaCl</td>
<td>30% NaCl</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VI</td>
<td>30% NaCl</td>
<td>seawater</td>
<td>180,363 ($\pm$ 5,759)</td>
<td>102</td>
</tr>
</tbody>
</table>
in 30 % NaCl resulted in essentially the same low activity as HgCl₂ treatment, indicating that 30 % NaCl effectively stopped microbial activity. However, when the sediment was allowed to recover for 15 min in seawater after 30 % NaCl pre-incubation, activity increased to 36 % of the seawater control. Preliminary experiments indicated that 1 to 2 h rest periods also resulted in 30 to 40 % recovery of the ¹⁴C-amino acid uptake activity (G. Lopez, unpubl.).

**Experiment 2**

Sediment pre-incubated in 30 % NaCl for 1.5 h, then transferred to seawater for 18 or 48 h, exhibited approximately the same ¹⁴C-amino acid uptake activity as samples kept throughout in seawater (Table 2). Sediment kept throughout in 30 % NaCl exhibited very low uptake.

**Experiment 3**

Sediment incubated in seawater took up more ¹⁴C-formaldehyde than sediment incubated in 30 % NaCl, at incubation temperatures of 35 °C or lower (Fig. 1). At higher temperatures, however, there was no difference between seawater and 30 % NaCl samples. The difference between seawater and 30 % NaCl treatments was greatest at intermediate temperatures.

![Fig. 1. Experiment 3: Effect of incubation temperature and 30 % NaCl on ¹⁴C-formaldehyde uptake](image)

**Experiment 4**

There was a significant positive relationship between ¹⁴C-formaldehyde uptake and organic content of the sediment samples (Fig. 2). This relationship could be complicated by saturated uptake by highly organic samples but non-saturated uptake by low-organic samples. If this was an important effect, then

![Fig. 2. Experiment 4: Effect of sedimentary organic matter on ¹⁴C-formaldehyde uptake](image)

**Experiment 5**

Sediment labelled for 44 h with ¹⁴C-formaldehyde, then transferred to seawater, lost ¹⁴C at ~ 0.2 % h⁻¹ over a 101 h period (Fig. 3).

**DISCUSSION**

Three lines of evidence suggest that most uptake of ¹⁴C-formaldehyde by sediment incubated in 30 %
NaCl is due to chemical reactions with sedimentary organic matter: (1) Positive correlation between $^{14}$C-formaldehyde uptake and organic content of sediment; (2) positive effect of temperature on uptake under NaCl incubation; (3) minimal uptake by ashed sediment. When microbes are not killed or de-activated, they account for most of the $^{14}$C-formaldehyde uptake under normal environmental temperatures. At temperatures of 60 °C and higher microbes are presumably killed, so chemical uptake predominates.

Microbes may account for much $^{14}$C-formaldehyde uptake because they actively take up formaldehyde, or because formaldehyde has a higher chemical affinity for microbes than for sedimentary organic matter. The results of the NaCl experiments demonstrate that the chemical uptake can proceed undisturbed, its characteristics make it an effective tool in radiolabelling methodology. Microbial activity is very effectively inhibited, but the inhibition appears to be largely reversible. Preliminary experiments indicate that microbes, after being subjected to 40 h of NaCl incubation followed by 24 h recovery in seawater, incorporated $^3$H-thymidine (see Hollibaugh et al., 1979). Because 30% NaCl does not appear to kill microbes, it probably also does not greatly alter the structure of the sedimentary organics, though its high ionic strength may affect sorption of dissolved and colloidal organics. This aspect has not been studied. NaCl is easily diluted from the labelled sediment, leaving no residue that might interfere with animals in feeding experiments. Of course, the $^{14}$C-formaldehyde itself may affect animals, but given the low concentrations needed to sufficiently label sediment ($\sim 10^{-5}$ M in this study), chemical bonding, and rinsings, it is unlikely that free formaldehyde concentrations in labelled sediments exceed $10^{-5}$ M. Many methods could be used to stop microbial activity and still allow chemical activity; few methods other than NaCl (or other inert solutes) incubation have reversible action on microbes, little effect on sedimentary organics, and can be completely rinsed from the labelled material.

Table 3. Experiment 4: Effect of sedimentary organic matter on $^{14}$C-formaldehyde uptake ($n = 2, \bar{x} \pm$ s.d.)

<table>
<thead>
<tr>
<th>Sediment</th>
<th>% organic</th>
<th>dpm mg$^{-1}$ dry wt</th>
<th>dpm mg$^{-1}$ organic matter</th>
<th>dpm mg$^{-1}$ ash wt (ashed sediments)</th>
<th>% uptake, ashed/dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.77</td>
<td>256.1</td>
<td>1,364</td>
<td>5.6</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>± 1.05</td>
<td>± 11.5</td>
<td>± 62</td>
<td>± 0.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16.85</td>
<td>429.5</td>
<td>2,535</td>
<td>9.7</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>± 0.49</td>
<td>± 3.7</td>
<td>± 21</td>
<td>± 6.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.38</td>
<td>147.1</td>
<td>2,735</td>
<td>5.6</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>± 1.05</td>
<td></td>
<td>± 115</td>
<td>± 2.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.04</td>
<td>70.1</td>
<td>1,383</td>
<td>4.5</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>± 0.53</td>
<td>± 9.9</td>
<td>± 62</td>
<td>± 0.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.46</td>
<td>5.3</td>
<td>1,140</td>
<td>0.3</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>± 0.01</td>
<td>± 1.1</td>
<td>± 244</td>
<td>± 0.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.39</td>
<td>7.1</td>
<td>1,832</td>
<td>0.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>± 0.06</td>
<td>± 2.4</td>
<td>± 633</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.09</td>
<td>2.6</td>
<td>2,808</td>
<td>0.1</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>± 0.01</td>
<td>± 0.1</td>
<td>± 71</td>
<td>± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Correct interpretation must be metabolic uptake. Microbes are effectively but reversibly inhibited by 30% NaCl; uptake of both $^{14}$C-mixed amino acids and $^{14}$C-formaldehyde under 30% NaCl incubation was very similar to uptake by killed samples. NaCl incubation also inhibits $^{14}$C-bicarbonate uptake in the light as effectively as HgCl$_2$ poisoning (G. Lopez and F. Carrasco, unpubl.). Reversibility of inhibition demonstrates that few microbes are killed by 30% NaCl treatment. Moreover, postincubation of $^{14}$C-amino acid labelled microbes in 30% NaCl did not result in greater loss of $^{14}$C than seawater postincubation (G. Lopez, unpubl.). Therefore, if inactive but live cells take up $^{14}$C-formaldehyde at the same rate as killed cells, higher uptake by active cells must be due to the metabolism of the cells, rather than their organic structure.

NaCl incubation is not only necessary for chemically labelling sedimentary organics with $^{14}$C-formaldehyde because it effectively inhibits microbial activity so
feeding, such as digestion and assimilation. We plea
osara (Montague). I. The assimilation of different compo-
tation to use this method to examine other aspects of
Kofoed, L. H. (1975a). The feeding biology of Hydrobia ven-
Hollibaugh, J. T
427-437
suitable for feeding experiments on deposit-feeders. Lirnnol. Oceanogr. 25: 172-181
The purpose of this study was to develop methods
Ashed sediments displayed much lower uptake of
14C-formaldehyde than did dried sediments, supporting
the idea that uptake was due primarily to reactions with organics. These results, however, should be viewed with some caution. High temperature ashing certainly altered the physical state of the clay minerals, so that dried sample may have displayed much higher uptake by undisturbed mineral grains. Experiments should be conducted using other methods of organic removal (peroxide treatment, low temperature ashing) to separate the organic and inorganic components of 14C-formaldehyde uptake. Certainly, inorganic adsorption of 14C-formaldehyde occurred, and it was easy to measure. Formaldehyde sticks to most everything, including baked glass fiber filters, and the methodological problems caused by adsorption were significant. Nevertheless, the experimental results are best explained by the conclusion that organic uptake was more important than inorganic uptake, and that high temperature ashing probably did not greatly reduce inorganic adsorption by sediments. High temperature alteration will affect clay minerals more than massive siliceous grains. In fact, our results indicate that ashing reduced 14C-formaldehyde uptake to 2.2 to 6.4% of dried uptake, regardless of sediment type (Table 3). If ashing had caused a serious artifact, it should have had a different effect on the high organic, muddy sediments (1-4) than on the low organic sands (5-7). That was not the case.

It is possible that formaldehyde does not react with sedimentary organics, but only adsorbs to it when the organics are themselves adsorbed to inorganics. For ingestion experiments, this distinction is academic; as long as the label is associated with the organic matter, and there is little change in activity during the course of a feeding experiment, the method can be used. The results of this study suggest that these conditions are met.

The purpose of this study was to develop methods suitable for feeding experiments on deposit-feeders. Using these methods, we are able to estimate ingestion rate and ingestion selectivity of sedimentary organic matter (Lopez and Cheng, 1982). There will be a temptation to use this method to examine other aspects of feeding, such as digestion and assimilation. We plea
that such experiments not be conducted until the nature of the formaldehyde-organic association is elucidated.

CONCLUSION

This study demonstrates that sedimentary organic matter is labelled when incubated with 14C-formaldehyde in 30% NaCl. The strong solute is an effective but reversible inhibitor of microbial activity. Microbial inhibition is necessary to allow chemical labelling because active microbes take up 14C-formaldehyde much more quickly than does chemical reaction with sedimentary organics. Chemical labelling of sedimentary organic matter meets the requirements necessary to conduct experiments on ingestion of this potential food source by deposit-feeders, such as studies on ingestion rate and ingestion selectivity (Lopez and Cheng, 1982). Adsorption of 14C-formaldehyde to particle surfaces appeared to account for only a small fraction of uptake by sediments, although this problem deserves further attention. The relationship between uptake and organic content suggests that certain classes of sedimentary organics dominate in sediments, or that formaldehyde has broad reactivity. Further study should include chemical fractionation of the labelled material.

Acknowledgements. We thank D. Capone, F. Carrasco, and E. Lopez for assisting in various aspects of this study, and several anonymous reviewers for critically reading earlier drafts. This work was supported by NSF grant (OCE8025345) to G. Lopez.

LITERATURE CITED


This paper was presented by Professor R. C. Newell; it was accepted for printing on March 3, 1982