PD-AAW-187

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 6/30/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-348
Third Progress Report

Attachment

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PD-AAW-287

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U.S.-ISRAEL COOPERATIVE DEVELOPMENT PROGRAM - CDR

Third Progress Report June 1987

Title of Research Project:

Staple Isotopes of Carbon, Nitrogen and Hydrogen as Naturally Occurring, Indigenous Tracers for Non-Invasive Studies in Human Nutrition (C5-348).

Grant Number: PDC-5544-G-SS-5097-00 (August 1985)

Principal Investigator:

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Cooperating Investigator:

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Institute of Chemistry, University of the Philippines, Los Banos, Laguna, Philippines.

Signature of Principal Investigator

Gerald L. Schroeder

OVERALL OBJECTIVES: evaluate the effectiveness of the ratios of 13C/12C (delta C), 15N/14N (delta N) and D/H (delta D) as they occur naturally in human foods as tracers in human nutrition.

OBJECTIVE OF THIRD SIX MONTHS: analyze samples taken in previous nutrition experiments; evaluate the data relative to the effectiveness of the three isotope pairs as tracers in human nutrition; plan additional nutrition experiments.

ACHIEVEMENTS

Sample Analysis

Samples taken in the preceding nutrition experiment have been analyzed. This experiment consisted of 12 adult subjects consuming either corn (delta C -9.7) or rice (delta C -27.0). In a cross-over design, six subjects first consumed boiled rice and six consumed boiled corn, for ten consecutive days. Then, following a six day break, the subjects reversed their diets and those who had consumed rice now consumed corn; those who had consumed corn now consumed rice. The cooked food was supplied in unrestricted quantities and multi-vitamin plus mineral tablets were given to each subject daily.

All sampling and first stage preparation of the samples was performed on-site, at Bigaa, Cabuyao, the test site. The actual isotope analyses were performed, as planned in the project format, using isotope separation mass spectrometers, in commercial laboratories located in the USA. The samples included hair, feces, urine and saliva taken each two days, starting with the first day and finishing with the last day. The only lack in samples was with feces. Saliva and urine were readily available from the subjects.

All samples were dried at 90 C upon collection. Two drops of concentrated HCl were added to the urine prior to drying. While this

prevented the loss of nitrogen by evaporation, it introduced an extraneous source of hydrogen and so delta D data are not reported for the urine.

Carbon dioxide for the delta C analyses was collected from the combustion of the sample at 575 C in the presence of copper oxide and silver wire. Hydrogen was collected as water vapor formed in the combustion for delta C. This water was reduced to hydrogen gas by reaction with zinc at 450 C. Alternatively, uranium was used to reduce the water to hydrogen. Residual adsorbed hydrogen on the uranium may cause a "memory" effect between samples. Therefore hydrogen analyses were performed in consecutive duplicates. The zinc was used for only one reduction and discarded. Therefore no memory effect was present. Nitrogen for delta N was collected from combustion of the sample at 850 C followed by a programmed reduction in temperature.

Representative data from these analyses are presented in Table 1. The values are referenced to PDB carbonate for delta C, atmospheric nitrogen for delta N, and SMOW (Standard Mean Ocean Water) for delta D.

Based on replicate analyses, one standard deviation of values in Table 1 is approximately 0.3 o/oo for delta C, 1.0 o/oo for delta N and 10 o/oo for delta D. We have not yet identified the causes of the unusually high variability for delta N and delta D analyses. Typically standard deviations for delta N and delta D are 0.5 o/oo and 5 o/oo, respectively.

Evaluation of Data

The isotopic compositions of the diets were well suited for the

goals of this experiment. Between the corn and rice there was a 17 o/oo difference in delta C and a 10 o/oo difference in delta N values. The difference in delta C results from the fact that corn is a C-4 type plant while the rice is a C-3 type plant. The difference in delta N resulted from different types of fertilization having been used with these two crops. The value of delta D varies considerably from crop to crop (see Table 1 of First Progress Report). Based on the range of opinions expressed in published research, there is no firm and comprehensive explanation of these differences. The delta D of the tap water at Bigaa (-56) is similar to values we have measured at other times in this general area (e.g., at UPLB approximately 20 km distant, water delta D ~-53).

The response of the subjects to the diets was variable. At first observation of the results, it might be inferred that occasionally extraneous foods were consumed during this period. However, the subjects assured us that only those foods and vitamins included in the tests were consumed. A careful inspection of the data reveals that for each subject, at least one of the sample categories responded to the diet. This indicated that the lack of response was due to a physiological, and not a dietary, excursion from the isotopic trend of the supplied food.

The most problematic response was that of subject Marivic, an un-married, 18 year old woman weighing 45.4 kg at the start and 44.5 kg after 10 days of the rice-only diet. Neither her saliva nor urine showed an isotopic response to the rice. In the other subjects, there was a clear, though not quantitative, response to diet by both of these sample categories. However Marivic's feces delta C values from

day 6 through day 10 matched her rice diet almost exactly, indicating her adherence to the experimental diet.

The fecal samples of subject Poleng, a 29 year old married male (54.5 kg on day zero and 54.5 kg on day 10) showed no delta C response to the corn diet although his urine and saliva delta C response was similar to that of the other subjects.

Among the three isotope pairs studied, only delta C was a predictor of the 10 day diet. The delta C values of saliva and urine agreed to within 1 o/oo for each subject. With the exception of subject Poleng, fecal delta C most clearly resembled the dietary intake. Although by day 4, fecal delta C matched dietary delta C for the rice diet, the equilibration of fecal delta C with corn was not reached during the 10 day period. The ease of eating rice relative to ease of eating corn is shown by the voluntary 50% greater consumption of rice in both 10 day periods relative to the consumption of corn. This higher intake likely affected the rate of fecal equilibration with diet. An increase in corn lignin (delta C -17) in the fecal matter can shift the feces to be approximately 1 o/oo more negative than the ingested total corn grain.

The delta N of the samples failed to respond to the diets in a recognizable manner within this 10 day period. Although the delta N value of the diet differed by as much as 14 o/oo from the initial delta N of the given sample category, there was no trend with time of sample delta N toward diet delta N. Nitrogen, being a component of protein, may have entered the samples via catabolism of previously stored body tissues. This explanation is not substantiated by the time zero values of delta N which, especially for the cumulative nature of hair protein, should be representative of the body store.

During this research period, we have made parallel studies of delta N as a tracer, working with lower animals. In that work, simple diets were kept constant during 3 to 10 fold weight gains by the animals (something quite impossible to demand of human subjects). The results showed that body delta N is approximately 4 o/oo more positive than diet delta N. The correlation was highly significant ($P < 0.0001$). However, the standard error of estimate for the regression equation of these data was 4 o/oo. A 4 o/oo error out of a total range of 10 o/oo in the delta N of foods reduces delta N to a marginal, qualitative tracer of dietary intake. In contrast, in that same work, muscle delta C was observed to be only 0.8 o/oo less negative than the diet. The standard error of estimate was 1 o/oo. A 1 o/oo error out of a total range of 20 o/oo in the delta C of foods makes delta C useful as a quantitative tracer.

The delta D values listed in Table 1 show no clear trend related to the diet. The data do not appear to be confounded by hydrogen from the drinking water. The subjects were all residents of the immediate area where the test was conducted. Therefore, their body water delta D, which reflects the drinking water delta D, did not undergo large changes during the time preceding and including the experimental period. Although some 20% of the hydrogen in protein is labile and subject to exchange with body water, both the absolute values and the trends of sample delta D vs time are not indicative of either inclusion or exclusion of the constant delta D water hydrogen in the pool of labile hydrogen. The 10 day period was insufficient to achieve an evaluation of the effectiveness of delta D as a nutrition tracer.

Future Experiments

The simple, one component diet places considerable strain on the subjects. Reluctance was expressed when a longer term experiment was suggested. Because equilibrium was not reached in a 10 day period, the next experiment is scheduled to last 20 days with the modification that after each 5 day period, one or two meals consisting of the same carbohydrate-rich staple plus a meat or fish supplement will be given. Sampling will concentrate on the days most remote from the consumption of the supplement. Although the goal of the experiment is to establish a non-invasive tracer system, taking blood will be included in the sampling regime (serum and RBC separately).

OUTLAYS TO DATE

To date we have spent $40,601.15 or 27% of our budget. In the next six months we anticipate increased expenses for isotope analyses and data processing.

Table 1. Representative values of delta C, delta N and delta D from selected body specimens taken during a feeding regime in which adults consumed either boiled rice or corn for 10 consecutive days.

DIETS		delta C	delta N	delta D			
Boiled corn		-9.7	-5.6	-105			
Boiled rice		-27.0	+4.8	-160			
Drinking water		----	----	-56			
SALIVA							
corn	day	delta C	delta N	delta D		delta C	delta D
subject Lito:	0	-21.2	+8.6	-86	Poleng:	-21.2	-76
	2	-19.0	+9.9	-80		-19.0	-76
	4	-18.2	+9.3	-88		-19.3	-71
	6	-17.6	+8.9	-91		-18.6	-84
	8	-16.8	+9.0	-90		-18.0	-92
	10	-16.4	+10.3	-102		-17.0	-95
Rice							
subject Delia:	0	-24.0	+9.7	-91	Marivic:	-19.8	-105
	2	-23.4	+11.4	-100		-20.8	-81
	4	-23.5	+12.1	-80		-21.9	-81
	6	-24.0	+10.2	-89		-21.6	-86
	8	-23.7	+12.0	-107		-22.3	-89
	10	-24.7	+11.5	-104		-21.9	-92
URINE							
Corn							
Lito:	0	-19.4	+8.9		Poleng:	-19.0	
	2	-19.0	+14.8			-18.0	
	4	-16.3	+8.5			-19.4	
	6	-17.4	+14.4			-16.3	
	8	-15.8	+1.5			-16.8	
	10	-15.7	+4.7			-16.0	
Rice							
Delia	0	-21.9	+4.1		Marivic:	-19.4	
	2	-23.3	+6.1			-19.3	
	4	-23.5	+7.3			-17.4	
	6	-23.6	+9.8			-18.5	
	8	-23.6	+6.7			-20.8	
	10	-24.1	+2.8			-20.9	
FECES							
Corn							
Lito:	0	-26.3	+7.6	-149	Poleng:	------	------
	2	-21.6	+7.4	-148		-17.9	-97
	4	-17.9	+6.7	-154		------	------
	6	-16.6	+7.8	-148		------	------
	8	-15.9	+4.9	-144		-17.0	-109
	10	-14.9	+4.6	-139		-19.8	-87
Rice							
Delia:	0	-25.8	+8.3	-118	Marivic:	-23.2	-106
	2	-26.8	+11.1	-117		-25.3	-114
	4	-27.3	+9.7	-93		------	------
	6	-27.1	+8.8	-109		-26.7	-107
	8	-27.1	+9.0	-99		-26.9	-104
	10	-27.1	+10.0	-94		-27.5	-114

Table 1 continued

HAIR

Corn	day	delta C	delta N	delta D		delta C	delta D
Lito:	0	-19.8	+9.9	-67	Poleng:	-20.1	-79
	4	-19.8	-----	-68		-----	-----
	10	-19.4	+9.2	-61		-19.7	-88
Rice							
Delia:	0	-22.0	+9.5	-85	Marivic:	-19.2	-71
	4	-22.4	+8.6	-73		------	------
	10	-22.4	+9.0	-76		-18.1	-67

Note: Standards are PDB carbonate for delta C; atmospheric nitrogen for delta N; SMOW (Standard Mean Ocean Water) for delta D. All values are o/oo.