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U.S.-ISRAEL COOPERATIVE DEVELOPMENT RESEARCH PROGRAM - CDR

Fourth Progress Report

December 1987

Title of Research Project:

Stable 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-5027-00 (August 1985)

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OVERALL OBJECTIVES are to 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.

OBJECTIVES OF THE FOURTH SIX MONTHS were to conduct the final experiments in which the subjects' diets would be limited to one or two selected components, and to evaluate the data obtained in these experiments. With this information we are to plan the experiments for the final 12 months of this project.

ACHIEVEMENTS

Nutrition Experiments

Two nutrition experiments were conducted in this period. The samples obtained in these experiments have been analysed for delta C, delta N and delta D. In both experiments, the foods were purchased prior to the start. This ensured that they originated in a single lot and so would have consistent isotopic values for the duration of the experiment.

Experiment number 1:

This experiment lasted 12 days. In this period, five free-living adults (four females and one male) consumed either milk plus rice or milk plus oatmeal as their primary food intakes for the twelve days. As such, milk was the major source of protein. The carbohydrate intake was mainly from the rice or oats, with a secondary source coming from the milk. Because both rice (delta C -25) and oats (delta C -27) are C-3 type plants, each subject was permitted one piece of fruit (also C-3 type plant) at each meal. This variety provided incentive to participate in the experiment for the otherwise hesitant volunteers. One off-the-shelf multi-vitamin plus mineral capsule and water ad liberatum were also provided daily to each subject.

In Table 1, the dietary intakes are listed. In the rice + milk diet, approximately 80% of the protein originated in the milk, while approximately 60% of the carbohydrate originated in the rice. As planned, the only other food consumed, the fruit, provided minor contribution to the total carbohydrate and protein intake.

In the oatmeal + milk diet, 2/3 of the protein originated in the milk and 1/3 in the oats (recall that at 14%, the protein content of oats is twice that of rice). Approximately 60% of the carbohydrate originated in the oatmeal and 30% in the milk

Isotope data related to the foods are also included in Table 1. These indicate that the grain and milk intakes are distinguishable by their 13C/12C ratios.

Our previous experiments did not provide a balanced nutritional intake. The single component was adequate for caloric needs but was deficient in protein. The present experimental diet provided both adequate caloric and protein intake. The provision of dietary protein reduced catabolic use of body tissues and so made the sampled body specimens more dependent upon the current dietary intake.

Representative values for the body specimens are listed in Table 2 according to specimen type. Comparisons of these isotopic values with those of the dietary components (Table 1) confirm, in our opinion, the tentative conclusions presented in our previous (third) progress report: of the three isotope pairs tested, delta C is the best predictor of the diet. Fractionation of the nitrogen and hydrogen isotopes results in an erratic relationship among delta N or delta D of body specimens and the delta N or delta D of the diets.

The poor relationship between delta N of the diet and the body specimens is in accord with results of experiments (ours and others) in which animals were grown on diets of constant and known delta N. The results of these works are shown in Figure 1. Although the delta N of body tissue is a function of the delta N of the food (probability of chance relation < 0.0001), the correlation coefficient (r squared) is only 0.6, and the standard estimate of error is 2.2 o/oo. Since the total range of delta N in foods is only approximately 10 o/oo, a spread of 4.4 o/oo in the certainty of prediction reduces delta N to the status of a qualitative indicator of diet. A least squares fit to the data intercepts the y-axis (delta N of muscle) at 4 o/oo. This demonstrates a fractionation which enriches 15N in the tissues relative to the 15N content of the diet. If the enrichment were constant, a correction could be made to account for this. The data of the current experiment and those data presented in Figure 1 indicate that the exact amount of enrichment of 15N, and hence the relation between diet and body specimen, is dependent upon the individual subject's metabolism.

For the body specimens sampled, delta D shows no correlation between diet and tissue. Since we had observed this in our previous experiments, only limited analyses of delta D were taken in this period. This poor correlation is paralleled in the results of an experiment (Table 3) in which we grew fish and shrimp on one of five diets.

Partition of the dietary components is indicated by the delta C data. Average urine delta C (-27.2) was 4 ± 2 o/oo less negative than the milk delta C, while average fecal delta C (-24.2 for rice + milk diet and -26.5 for oatmeal + milk diet) was 0.9 ±0.5 less negative than the cereal component of the diet. Average saliva delta C (-21 ±0.1 o/oo for rice + milk and -19.8 for oatmeal + milk) values were within 1 o/oo of the milk delta C.

The lack of trend to the delta C data of these specimens vs date of sampling might be interpreted as an insensitivity of saliva delta C to diet delta C. However, note the data in our second and third progress reports where ten days of a corn-only regime resulted in the delta C of saliva changing by 4 o/oo toward the value of the corn.

The similarity of the delta C of serum to that of the red blood cells (RBC) may indicate the similarity of protein delta C of this diet to the average protein delta C normally eaten. The life of an RBC is 120 days and so would not be significantly responsive to a diet of twelve days duration. The serum delta C would be expected to respond rapidly to the dietary change.

Experiment number 2:

This experiment lasted 20 days. In this period, 3 free-living adults consumed either rice or corn ad liberatum. A multi-vitamin plus mineral capsule and unlimited water were also provided daily. Once each 4 days, after sampling scheduled for that day, the subjects were given one meal containing varied protein sources (meat or fish). During the experiment, one of the volunteers complained of discomfort and constipation and so has been dropped from the experiment.

The data related to the isotopic composition of the foods and body specimens are listed in Table 4. Comparisons of these data with those included in our third progress report show that transient equilibrium in the fecal delta C for the rice diet is reached within 4 days, while with corn, approximately 16 days were required. The exception to this is the one "corn-only" case reported in our second report wherein equilibrium was also reached in four days. In that case, the rate of corn consumption was two times greater than in the other experiments.

The fact that with rice and oatmeal the fecal delta C consistently comes to within 1 o/oo of the food delta C, while with corn, the fecal delta C is consistently 4 to 6 o/oo more negative than the diet indicates a varied delta C composition of the corn and fractionation of components during digestion. By separating the corn into its fiber and non-fiber components, we have found that corn lignin is 5 to 7 o/oo more negative than the total corn grain and that total corn solids are 1 to 3 o/oo more negative than the corn solubles. These observations account for much of the shift in fecal delta C relative to the diet delta C in this feeding regime.

The values for saliva indicate a mixed contribution from body store and from the immediate diet. The saliva delta C value of -16 has been observed in all the corn-only tests to date. The delta C of saliva on the rice-only diet (-23) was also observed in our previous rice-only diets, but is different from the value obtained in the rice + milk experiment reported here in Table 2. This difference reflects the strong contribution which milk makes to the active protein pool. The same type of distribution is seen in the delta C values of urine for the several dietary conditions tested to date. On a protein-poor diet (rice-only or corn-only) the delta C of urine approached the delta C of the diet, but contributions from body store are evident. When milk is added to the foods, the urine delta C showed a strong contribution from the milk.

FUTURE EXPERIMENTS

It appears that 20 days is adequate to reach transient equilibrium in the isotopic composition of the specimens of interst. We are planning one more background experiment in which, for 20 days, volunteers will consume a 2-component, protein plus carbohydrate diet. The components will be selected so that half the subjects will receive a diet in which protein and carbohydrate delta C values are similar. Half the subjects will be given foods in which the main protein and carbohydrate source will have measureably different delta C values. This approach should help clarify the sources of compounds found in the body specimens samples, especially those of the urine, saliva and blood serum.

OUTLAYS TO DATE

To date we have spent $77,045.69 or 51.4% of our budget. The political unrest in the Philippines has caused a few delays in the planning of the experiments and therefore in some of the planned outlays.

Table 1. Components of eleven day controlled rice plus milk and oatmeal plus milk diets.

Component	Isotopic value delC delN delD	Subject's daily consumption	Protein amount	%	Carbohydrate amount	%
rice + milk diet						
boxed milk (1% fat)	-21.5 +5.3 -30	1.5 liters	61.0 g	76.8%	102 g	29.4%
(fat-free	-20.8)					
rice (dry)	-25.3 +5.6 -48	250 g	17.5 g	22.0%	200 g	57.6%
fruit (dry)	-26	48 g	0.9 g	1.1%	45 g	13.0%
total			79.4 g	99.9%	347 g	100%
oatmeal + milk diet						
Powdered fat-free milk	-20.7 +7.3 +7	210 g	75.0 g	67.5%	109 g	33.3%
oatmeal (dry)	-26.9 +5.3 -63	250 g	35.6 g	32.0%	188 g	57.5%
fruit (dry)	-26	32 g	0.5 g	0.5%	30 g	9.2%
total			111.1 g	100%	327.0g	100%

Note: All delta C values are relative to PDB carbonate; delta N values are relative to atmospheric nitrogen; delta D values are relative to Standard Mean Ocean Water (SMOW).

Table 2. Representative values of delta C, delta N and delta D from selected body specimens taken during feeding regimes in which adults consumed either rice plus milk or oatmeal plus milk for eleven consecutive days (see also Table 1).

SALIVA

	day	delta C	delta N	delta D
rice + milk				
subject # 1	2	-20.4	+9.2	-66
	8	-20.8	+7.8	
	12	-21.2	+9.7	-73
subject # 2	3	-21.2	+8.3	
	12	-21.0	+10.3	
subject # 3	3	-18.9	+9.2	-73
	12	-21.0	+8.6	-78
subject # 4	3	-20.2	+8.9	
	8	-21.3	+8.9	
	12	-20.6	+9.3	
oatmeal + milk				
subject # 5	1	-19.3	+10.6	-48
	8	-19.6	+9.9	
	12	-19.8	+8.7	-46

URINE

	day	delta C	delta N	delta D
rice + milk				
subject # 1	2	-21.6	+6.3	+23
	8	-17.7	+1i.9	
	12	-17.6	+16.0	-13
subject # 2	2	-18.7	+12.6	
	12	-16.7	+12.6	
subject # 3	2	-19.0	+14.5	-47
	12	-i8.6	+12.9	+2
subject # 4	2	-18.8	+10.4	
	8	-17.3	+7.7	
	12	-15.0	+20.8	
oatmeal + milk				
subject # 5	1	-18.1	+10.6	+15
	8	-18.8	+9.2	
	12	-18.2	+10.3	-15

Table 2 continued

	day	delta C	delta N	delta D
FECES				
rice + milk				
subject # 1	3	-25.0	+4.5	-98
	9	-25.2	+5.8	-106
subject # 2	2	-24.6	+7.0	
	10	-24.6	+9.2	
subject # 3	2	-24.8	+6.7	-136
	12	-24.1	+6.8	-114
subject # 4	12	-23.3	+8.9	
oatmeal + milk				
subject # 5	1	-24.4	+5.0	-107
	4	-27.2	+5.6	
	8	-27.4	+7.4	
	12	-26.5	+7.0	-121
BLOOD SERUM				
rice + milk				
subject # 1	2	-20.8	+10.5	-50
	12	-20.9	+11.3	-42
subject # 2	2	-20.6	+10.4	
	12	-21.4	+10.9	
subject # 3	2	-21.2	+10.2	-34
	12	-20.6	+10.5	-39
subject # 4	12	-20.6	+9.9	
oatmeal + milk				
subject # 5	4	-20.7	+11.3	-54
	8	-20.5	+8.9	
	12	-20.9	+9.1	-50
RED BLOOD CELLS				
rice + milk				
subject # 1	2	-20.4	+7.9	-62
	12	-20.7	+8.1	-60
subject # 2	12	-20.9	+7.4	
subject # 3	12	-19.6	+6.7	-40
subject # 4	2	-20.3	+7.5	
	12	-20.0	+7.5	
oatmeal + milk				
subject # 5	4	-19.9	+6.9	-45
	8	-20.0	+8.3	
	12	-19.9	+6.6	-39

Table 3. Delta C and delta D values of fish and shrimp muscle fed one of five diets.

	delta C	delta D
Diet: corn meal	-11.1	-14
muscle	-10.0	-74
Diet: corn grain	-12.0	-28
muscle	-11.7	-44
Diet: oatmeal	-26.8	-60
muscle	-25.0	-161
Diet: white rice	-26.3	-44
muscle	-24.2	-130
Diet: egg yolk	-19.4	-123
muscle	-17.3	-60

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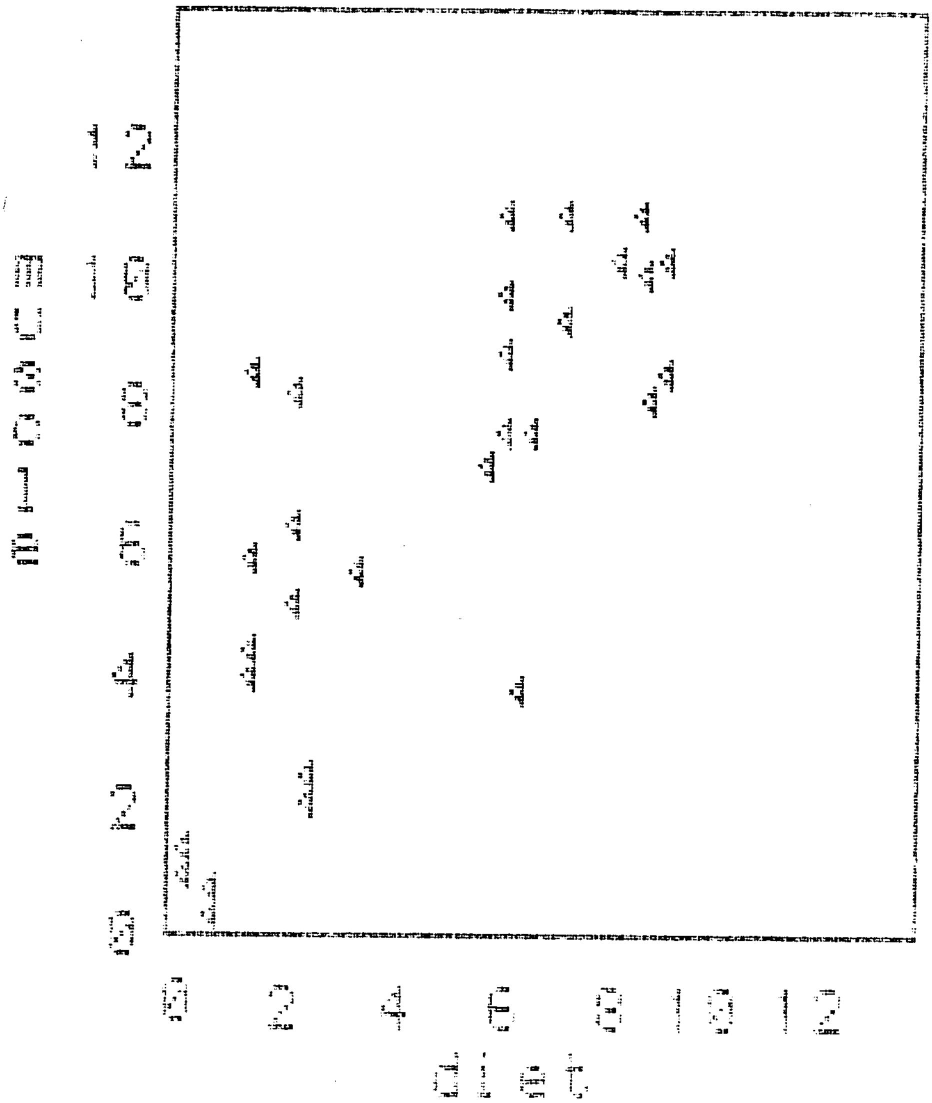
Table 4. Representative values of delta C, delta N and delta D from selected body specimens and dietary components during feeding regimes in which two adults consumed either boiled rice or corn as their main food for twenty days.

Diet	delta C	delta N
boiled corn	-10.5	-1.1
boiled rice	-27.5	+4.8

		corn diet		rice diet	
	day	delta C	delta N	delta C	delta N
saliva	0	-21.8	+10.2	-22.5	+9.5
	16	-18.0	+9.3	-23.5	+9.3
	20	-16.2	+9.8	-23.2	+9.2
urine	0	-22.0	+7.3	-21.7	+6.9
	16	-16.9	+6.1	-22.5	+7.1
	20	-17.7	+5.2	-22.8	+5.5
feces	0	-25.8	+6.8	-24.7	+6.0
	16	-16.9	+4.3	-26.6	+6.3
	20	-15.6	+3.4	-26.6	+5.1
hair	0	-20.2	+10.0	-20.8	+9.5
	8	-17.6	+9.3	-21.4	+9.5

delta N

Figure 1 muscle vs diet



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U.S.-ISRAEL COOPERATIVE DEVELOPMENT RESEARCH PROGRAM - CDR

Fifth Progress Report June 1988

Title of Research Project:

Stable 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)

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OVERALL OBJECTIVES are to 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.

OBJECTIVES OF THE FIFTH SIX MONTHS: Primary: to define the accuracy with which delta C can be used as an indicator of the diet being consumed and that part of the diet which is being assimilated or excreted; Secondary: to evaluate delta N as a qualitative indicator of dietary protein.

ACHIEVEMENTS

Nutrition Experiments

Four nutrition experiments were conducted in this period. These tests used a variety of components in an attempt to isolate the primary sources contributing to the body tissues which we sample. The duration of the experiments ranged from three days to three weeks. In several tests breath was monitored in addition to the body tissues sampled in the past.

In summary, the results of the experiments of this period demonstrate that the delta C values of urine, saliva and blood serum are predictors of the assimilated proteins, provided that a correction is made for inclusion of catabolized material in new tissues. Approximately 30% of the metabolically active pool of amino acids is taken from recently consumed foods and approximately 70% is taken from recycled or catabolized body tissue. When the diet is constant over long periods, such as in animal husbandry where five to ten fold weight gains are possible on a single diet, then both current diet and catabolized tissues have the same delta C value. In such cases the match between diet delta C and muscle delta C is excellent. The muscle delta C averages 0.8 o/oo less negative than the diet delta C, with a 95% confidence interval of less than 1 o/oo. We observe this relationship over the entire range of dietary delta C values (Figure 1).

When the diet is changing, as in the case of free living humans, the contribution of the body pool delta C value becomes the dominant term in determining the delta C of the newly formed tissues. The inclusion of both of these terms (0.7 x body pool delta C + 0.3 x recently consumed food delta C) predicts the delta C values of blood serum, saliva, and urine to within 2 o/oo for data taken in this project, provided that the diet has been constant for twenty days. An infant consuming only breast milk is also subject to changes in dietary delta C since breast milk delta C reflects the mother's changing diet (Table 1 in our First Progress Report).

The relation between delta N of the diet and that of the tissues sampled is less exact. Even with corrections for the 4 o/oo fractionation that exists between the diet delta N and the assimilated delta N (see our Fourth Progress Report), and for the 3:7 ratio in contributions of newly assimilated foods vs catabolized tissues, delta N remains a qualitative, not quantitative indicator of assimilated foods.

Experiment 1:

In this experiment, for ten days, free living adults consumed diets restricted to C-3 foods. The main protein came from purified soy protein and peanuts, or from full cream powdered milk of Australian origin and peanuts. Australian milk was chosen because the cows there receive a pure C-3 diet. This produces a milk with a delta C value of -29.3, containing casein with delta C -28.5. This highly negative value is in contrast with milk of U.S.A. origin. In US milk, the mixed C-3 + C-4 diet of dairy herds produces a delta C value in the milk of appoximately -19 for powdered skim milk.

In both the soy-based and milk-based diets, C-3 fruits, vegetables and wheat bread were permitted. These had a delta C value of approximately -26.

Results for this experiment are listed in Table 1. Based on the 3:7 distribution between recently ingested foods and catabolized tissues, the predicted delta C of saliva, serum and urine in the soy diet is -21.7. This is in close agreement with the values measured on days #5 and #10 (-21.5, -21.2, -22.2, for saliva, serum and urine, respectively, observed on day 10). The data obtained from the diet in which milk supplied the main protein have the same good match between calculated and measured values.

The similarity of fecal delta C for the two tests demonstrates the similarity of the average isotopic composition of these two diets.

Experiment 2:

The data from this experiment are limited to isotopic values of blood serum. Free living adults consumed either rice or corn (plus water and vitamin/mineral tablets) for ten or twenty days. The results are listed in Table 2. The calculated equilibrium values for delta C of the serum are -24 for the rice diet and -19 for the corn diet. For the twenty day diets, there is good agreement between the observed and calculated values. The absence of baseline (day #0) data for the 10 day test makes interpretation of the data difficult.

As in the experiments of the previous report periods, the delta N and delta D values do not follow consistent trends. The 4 o/oo

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fractionation of delta N is only an approximation and may vary depending upon the availability of dietary protein. This may be the cause of the weak manner in which delta N tracks changes in the diet. The multiple sources of hydrogen available to the free living test subjects make definition of the dietary hydrogen intake impractical.

Experiment 3:

Delta C of exhaled carbon dioxide should be an indicator of foods metabolized for energy. In preliminary experiments during which only C-3 foods were consumed for 5 days, we observed close agreement between diet and breath delta C values, both being approximately -26 o/oo.

To measure the short term response of breath delta C to dietary changes, a subject, whose normal diet is rich in C-3 type foods, consumed only corn (C-4) for 24 hours, then switched to a C-3 diet (mixed rice, mungbean, orange) and then, on day #3, ate corn plus mungbean. Breath was sampled at varied intervals during the test and was analysed for delta C at the CNRC by S. Sekely. The results of this test are listed in Table 3.

It is only on day #3 that any evidence of the C-4 component becomes evident in the breath. By this time, the diet had changed to include C-3 foods and so the full impact of the C-4 component was muted.

The data indicate that two or more days of constant diet are required for breath to be an indicator of consumed foods.

This slow response of breath delta C to the diet is surprising. Readily digestible foods would seem to be rapidlv available to the metabolic processes and so this experiment warrants repetition.

The slow response of breath, serum and saliva to diet is in marked contrast to the rapid response of breast milk to dietary changes. The data reported in our First Progress Report are typical of the many studies we have made of lactating women. Six hours after changing the carbohydrate of the woman's diet from C-3 (delta C -25) to C-4 (delta C -12), the delta C of her milk lactose had changed from -23.5 to -17.6. Thirteen hours after the diet change, the lactose delta C was -15.5. We see that lactose formation is almost immediately repsonsive to the consumed food. The protein component of the milk also changed, but by 3 o/oo. This is impressive since the protein-rich foods of her diet (milk, eggs, meat) were not changed.

Experiment 4:

This experiment is the most extensive of this six month reporting period. For ten days, subjects consumed one of the following diets: corn only (C-4), corn plus mungbean (C-4 + C-3), rice plus mungbean (all C-3), corn plus Australian milk (C-4 + C-3). Samples of blood serum, saliva, urine, feces and breath were collected throughout the test periods. The samples are currently being processed for delta C analysis. The data will provide a comparison of the assimilation of mungbean. a common local protein source, with that of milk, a high qualtiy protein.

CONCLUSION

Isotopicly speaking, it is popular to say: "You are what you eat." It seems that this must be modified to read : "You are what you ate." The delta C response of tissues we have measured is slow (weeks to reach transient equilibrium) and then it is heavily influenced by the existing body composition, i.e., what you ate, cumulatively, over the past months or years. We have found that only breast milk (lactose and protein) and excrement (urine and feces) change rapidly and in accord with the recently consumed diet.

The long term response of populations to dietary norms is evident from studies we and others have made. We have found that hair from individuals living on the east coast and the mid-west of the U.S.A. is similar, approximately -16.5. This reflects the C-4 input of corn-fed cattle and poultry (-14) and the mixed C-4 and C-3 feed of dairy herds (powdered skim milk from California, Florida and Pennsylvania had delta C values of -19, -19 and -18, respectively). The foods of Wuxi, China are imaged in the delta C of local inhabitants hair (-22) and in the delta C of their foods (pig -28, fish -22, milk -21). In Israel, our hair has a delta C value of approximately -18, our protein-rich foods -22 (dairy) and -16 (poultry).

From the results to date, it is becoming clear that delta C is a useful tracer only when good baseline data are available and the diet is controlled during an experimental period of at least a week.

FUTURE EXPERIMENTS

During the final period of this project we will emphasize studies of assimilation by lactating women and of a diet combining corn and fish. These results in combination with those of Experiment 4, described above, relate directly to the nutrition needs of populations at risk for malnutrition.

OUTLAYS TO DATE

To date we have spent $103,831.24 or 69.2% of our budget. The political activity in the Philippines has continued to cause some delays in the planning and implementation of the experiments and therefore in some of the planned outlays. Because this research is not based in a major metropolitan center, communications are less rapid than we would desire.

Table 1. Isotopic values for selected body tissues sampled while adult males consumed pure C-3 diets for ten days and then consumed C-3 + C-4 foods for 2 days.

DIET	delta C	delta N
soy protein	-24.6	+0.3
vegetables and oats	-26	
bread	-25	

	delta C (PDB, o/oo)				
	day 0	day 5	day 10	//	day 12
red blood cells	-21.0	-21.0	-21.6	//	-21.2
blood serum	-20.7	-21.1	-21.2	//	-21.1
saliva	-20.0	-21.2	-21.5	//	-19.9
urine	---	-21.4	-22.2	//	-17.8
feces	---	-26.4	-27.4	//	-25.9

	delta N (atmospheric nitrogen, o/oo)				
	day 0	day 5	day 10	//	day 12
red blood cells	---	+8.5	+7.3	//	7.4
blood serum	---	8.7	8.4	//	9.0
saliva	---	6.7	6.1	//	6.9
urine	---	6.1	4.9	//	9.4
feces	---	5.0	3.5	//	4.6

DIET	delta C	delta N
powdered milk	-29.3	+3.5
" " casein	-28.5	
" " fat	-31.5	
vegetables and oats	-26	
bread	-25	

	delta C (PDB, o/oo)						
	day 0	day 2	day 4	day 7	day 10	//	day 11
RBC	-20.5	-20.5	-20.6	-20.5	-20.6	//	-20.8
serum	---	-20.8	-20.7	-21.4	-21.5	//	-21.3
saliva	-19.6	-19.7	-20.9	-20.8	-20.8	//	-20.3
urine	---	-21.2	-22.4	-22.3	-22.5	//	-19.1
feces	---	-26.4*	-26.9	-27.2	-26.7	//	-24.2
shavings	---	-19.7	-19.6	-19.8	-19.6	//	-19.7

*This fecal sample was taken on day #3.

	delta N (atmospheric nitrogen, o/oo)						
	day 0	day 2	day 4	day 7	day 10	//	day 11
serum	---	+7.3	+6.6	+6.8	+9.9	//	---
saliva	---	6.4	3.3	3.8	4.8	//	4.6

note: Acidifying samples prior to analysis had no effect on the delta C or delta N value. Acidification removes carbonate-carbon which may be present and retards volatilization of ammonia-nitrogen.

Table 2. Isotopic values for blood serum while adults consumed only corn (C-4) or rice (C-3) during the periods indicated.

DIET	delta C	delta N	delta D
boiled corn	-10	-6	-105
boiled rice	-27	+5	-160

Corn diet	delta C			delta N			delta D		
day:	0	10	20	0	10	20	0	10	20
	---	-21.2	---	--	+9.3	--	--	-82	--
	---	-19.9	---	--	10.0	--	--	-86	--
	-21.4	-20.1	-19.6	10.6	9.6	9.4	-95	-78	-89
	-20.3	-19.6	-19.7	10.0	9.8	10.1	-83	-76	-83
	-20.3	-17.7	---	10.0	9.4	--	-83	-81	--

Rice diet	delta C			delta N			delta D		
day:	0	10	20	0	10	20	0	10	20
	---	-21.7	---	--	+9.9	--	--	-84	--
	---	-21.7	---	--	8.8	--	--	-85	--
	---	-21.6	---	--	9.5	--	--	-80	--
	-23.0	-22.5	-23.3	9.3	10.7	9.4	-83	-91	-85

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Table 3. Delta C values of breath sampled while subject consumed a diet containing only C-4 or C-3 or a mix of C-4 and C-3 foods.

Day #1
Corn (C-4)

time	delta C
0800	-23.4
0800	-22.1
0920	-22.3
1000	-23.3
1100	-23.3
1300	-24.0
1600	-24.2

Day #2
Mixed C-3 (rice, mungbean, fruit)

time	delta C
0800	-24.2
1300	-23.4

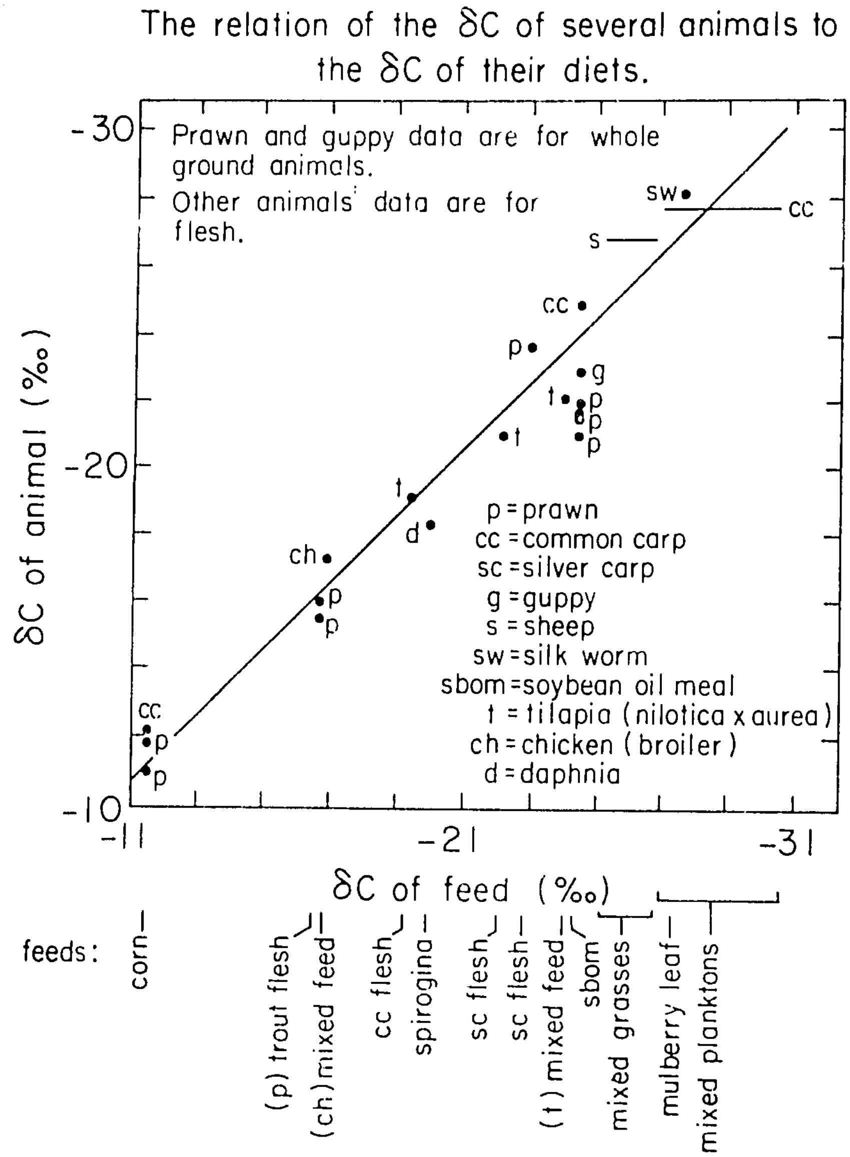
Day #3
Corn + mungbean

time	delta C
0800	-22.5
0800	-24.9
0900	-24.6
0920	-21.0
1230	-21.1
1250	-20.9
1310	-20.5
1410	-16.9
1610	-21.3

Day #4
Mixed C-4 + C-3

time	delta C
1300	-22.2
1300	-19.1
1300	-20.9
1300	-19.7

Figure 1



This figure is adapted from: Schroeder, G. (1987) Carbon and nitrogen budgets in manured fish ponds on Israel's coastal plain. Aquaculture 62:259-279.