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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).

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Rec'd in DC JUN 10 1988

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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 -25.

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 rapidly 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	-26.5	
" " 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.

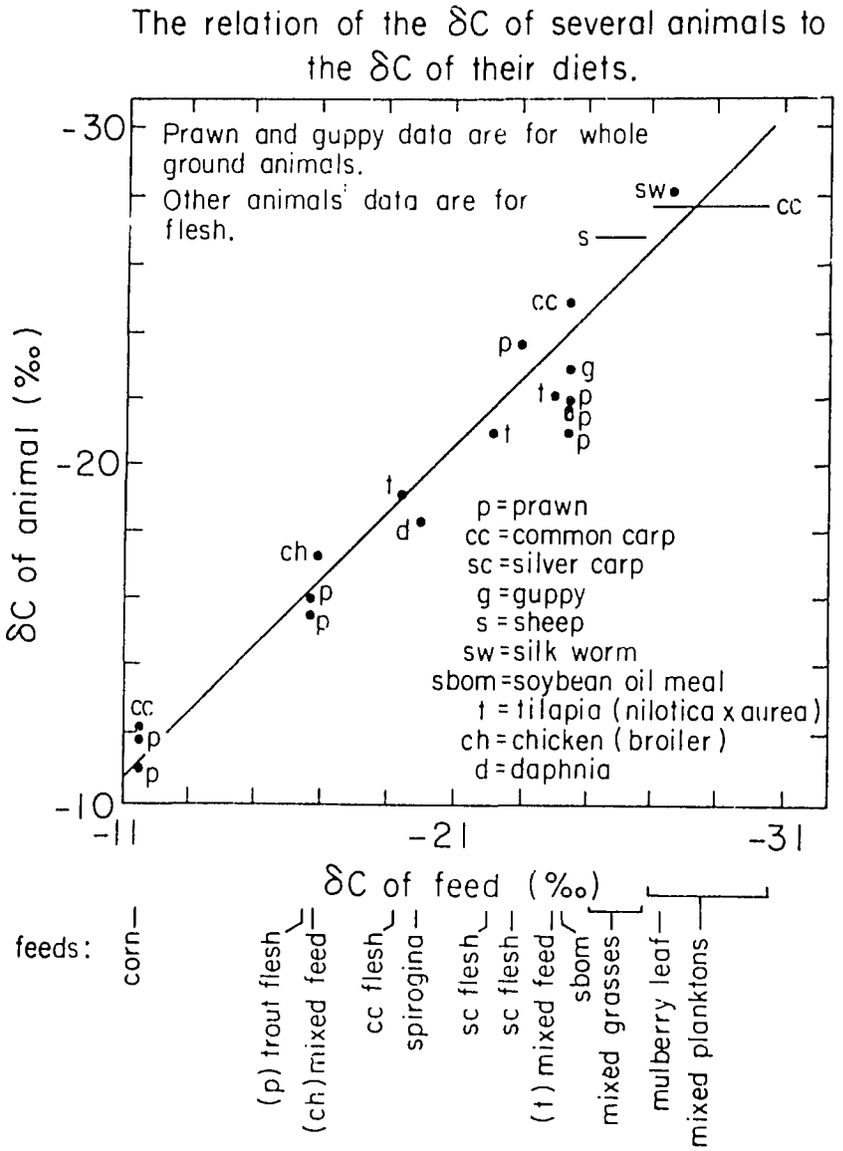
DIET	delta C	delta N	delta D
boiled corn	-10	-6	-105
boiled rice	-27	+5	-160

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

xxxx

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.