

AGENCY FOR INTERNATIONAL DEVELOPMENT  
WASHINGTON, D.C. 20523

DATE: 1/6/89

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. C 5 - 348  
6th PR Dec 88

Attachment

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

Sixth Progress Report

December 1988

Title of Research Project:

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

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

Principal Investigator:

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Cooperating Investigator:

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Signature of Principal Investigator:

*Gerald L. Schroeder*

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Rec'd in SCI: DEC 28 1988

OVERALL OBJECTIVES are to evaluate the effectiveness of the ratios of  $^{13}\text{C}/^{12}\text{C}$  ( $\delta\text{C}$ ),  $^{15}\text{N}/^{14}\text{N}$  ( $\delta\text{N}$ ) and  $\text{D}/\text{H}$  ( $\delta\text{D}$ ) as they occur naturally in human foods as tracers in human nutrition.

OBJECTIVES OF THE SIXTH SIX MONTHS were two-fold. The primary objective was to measure the isotopic correlation between breath carbon dioxide and the current diet. The second objective was to measure in nursing mothers the isotopic correlation between protein, lactose and lipid fractions of the breast milk and the consumed diet.

### ACHIEVEMENTS

#### Nutrition Experiments

##### Experiment 1:

To identify the relation between the  $\delta\text{C}$  of exhaled carbon dioxide and the ingested diet  $\delta\text{C}$ , 10 healthy, free living adults consumed for 20 days diets ranging from an extreme of corn-only (that is, the only food for the 20 days was corn having a  $\delta\text{C}$  of  $-11$  o/oo) to a balanced diet of rice plus fish with small amounts of C-3 type vegetables for variety. All subjects were given multi-vitamins and mineral capsules daily and were allowed water ad liberatum. Breath was sampled by having the subject exhale into (and in doing so inflate) a collapsed plastic bag. Twenty cc of breath were at once drawn from this bag into a Vacutainer. This system of sampling was developed at the Baylor College of Medicine in Houston. Dr. Librojo studied the method during her visits there.  $\delta\text{C}$  of the breath was analysed by freezing the breath carbon dioxide directly into the line of an isotope separation mass spectrometer.

The actual diets and the  $\delta\text{C}$  data obtained in this experiment are shown in Table 1 and in Figure 1. Several aspects of the data are worthy of comment.

The reproducibility is in general excellent. Duplicate samples

usually differed by less than 0.5 o/oo.

When the diet was nutritionally unbalanced, as in the corn-only diet which was deficient in protein, there was a strong dependence of the body on catabolized tissue. This is seen by the large differences (as much as 2 o/oo) between breath delta C collected before breakfast (after a 12 hour night-time fast) and before lunch. In the balanced diets (rice plus milk, and rice plus mungbean), the delta C difference between before breakfast samples and those samples collected at lunch time was less than 0.5 o/oo.

Although the delta C of breath obtained in the rice plus fish diet differed statistically from that of the breath collected in the rice plus milk diet, the average difference was small (less than 1 o/oo) and much less than the difference between the delta C values of the protein sources (fish delta C -23; milk delta C -26) of these two diets. The carbohydrate, which was rice (delta C -26) in both diets, apparently was the main source of the exhaled carbon dioxide. In the corn-only diet, the corn (delta C -11) was not the main source of breath carbon dioxide (delta C -18.4 to -21.9). This was approximately equally shared between body stores (which produced a breath delta C of -23 to -24; see delta C data for all of the day #0 breath tests taken in the fasting subjects before breakfast) and the current corn diet (delta C -11).

#### Experiment 2:

Three healthy adult, free living, nursing mothers consumed diets of either rice (delta C -26.9; delta N +4.0) plus mudfish (delta C -22.7; delta N +13.0) plus small amounts of C-3 type vegetables, or corn (delta C -11.0; delta N +3) plus mungbean (delta C -26.1; delta N -1.0) for 20 consecutive days. This diet format provided the main protein and the main carbohydrate from 2 separate sources. Multi-

vitamins and mineral capsules were taken daily. Water ad liberatum was provided.

The results of this experiment are listed in Tables 2 and 3.

Three fractions of breast milk were separated by centrifuge. All 3 fractions show the strong influence of the carbohydrate of the diet even in the presence of a good protein such as fish and mungbean. It is of interest that in the corn plus mungbean diet, the delta C of the milk protein was only 1 o/oo more negative than the delta C of the milk lactose even though the main dietary protein was 15 o/oo more negative than the dietary carbohydrate. Both the lactose and the protein delta C values of the milk indicate a preferential assimilation of the diet carbohydrate over the diet protein into the protein and lactose breast milk fractions.

Delta N data from these feeding trials are considerably less consistent than the delta C data. Although in studies with controlled diets and laboratory animals, the muscle delta N tends toward being 4 o/oo more positive than the diet delta N (that is, the muscle is enriched with  $^{15}\text{-N}$  relative to the diet), the breast milk protein delta N showed no such trend unless the carbohydrate-rich component (the rice or the corn, respectively for the 2 diets) was the main nitrogen source. In the corn plus mungbean diet, the delta C data indicated a variability in the lactose and protein dependence upon the corn and the mungbean. The delta N data indicate an almost continual dependence upon the corn as the nitrogen source. This was similar to the delta N data of the saliva and the blood serum.

We are well aware of the use of delta N as a tracer in nutrition studies. In that work, for instance the large number of studies reported by Baylor College of Medicine in Houston, the  $^{15}\text{-N}$  is usually added as an enriched tracer. In such cases, the  $^{15}\text{-N}$  takes on

the role of a separate tracer and differs from the use of  $^{15}\text{-N}$  in its natural abundance. We have seen no controlled studies reported which identify  $^{15}\text{-N}$ , in its natural abundance, as a tracer with a fidelity better than that shown in our fourth and fifth progress reports; namely: a fractionation that results in an approximate  $4\text{ ‰} \pm 2\text{ ‰}$  increase in  $\delta\text{N}$  in going from diet nitrogen to newly formed tissue nitrogen, and a correlation coefficient of only 0.6 for the relationship between diet and tissue  $\delta\text{N}$ . This standard deviation is a large fraction of the total range in naturally occurring  $\delta\text{N}$  (approximately  $10\text{ ‰}$ ). The correlation coefficient shows only a weak relationship between diet and tissue  $\delta\text{N}$  values.

#### FUTURE EXPERIMENTS

During the final period of this project we will study remaining samples taken from nursing mothers in an attempt to better understand the relationship between ingested foods and the assimilation of these foods into breast milk.

#### OUTLAYS TO DATE

To date we have spent \$132,974.67 or 88.6% of our budget. The six month extension granted to this project will not require expenditures which exceed the total budget.

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Table 1 delta C of carbon dioxide in breath collected from subjects consuming the indicated diets.

| day #                         | 0     |       | 1     |       | 2     |       | 5     |       | 10    |       | 15    |    | 17    |       | 20    |       | kg food eaten |       |       |       |                             |
|-------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|-------|-------|-------|-------|---------------|-------|-------|-------|-----------------------------|
|                               | BB    | AB    | L     | S     | BB    | S     | BB    | AB    | BL    | BB    | AB    | BL | S     | BB    | L     | BS    |               | BB    | BL    | L     | BB                          |
| corn<br>(Randy)               | -23.4 |       | -21.8 | -21.6 | -23.3 |       | -21.7 |       | -19.4 | -20.9 | -19.0 |    |       | -20.2 | -17.6 |       | -16.5         | -18.4 |       | -18.5 | 38.1 corn                   |
|                               | -23.7 |       |       |       |       |       | -21.7 |       |       | -20.8 |       |    |       | -20.3 |       |       |               |       |       |       |                             |
| corn<br>(Loreng)              | -23.2 |       |       |       |       |       | -21.7 |       |       |       |       |    |       |       |       |       |               |       |       | -21.4 | 38.4 corn                   |
|                               | -23.6 |       | -22.8 |       |       |       |       |       |       | -22.7 |       |    |       | -21.5 |       |       |               |       |       |       |                             |
| corn +<br>mungbean<br>(Lito)  | -23.9 | -21.8 | -19.2 | -21.3 | -22.3 |       | -20.4 |       |       | -19.9 | -19.0 |    |       | -19.2 | -19.5 |       |               | -14.9 | -18.7 |       | 40.9 corn<br>11.8 mungbean  |
|                               | -24.4 | -23.0 |       |       |       |       | -20.8 |       |       | -18.0 | -20.3 |    |       |       | 18.4  |       |               |       | -19.2 |       |                             |
| corn +<br>mungbean<br>(Layda) | -23.2 | -22.8 | -20.0 |       |       |       | -15.2 | -15.9 |       | -16.8 | -16.9 |    |       | -17.5 | -17.6 |       | -14.9         |       | -14.9 |       | 34.8 corn<br>22.1 mungbean  |
|                               | -22.6 |       |       |       |       |       |       |       |       |       |       |    |       |       |       |       |               |       |       |       |                             |
| rice +<br>mungbean<br>(Lilly) | -24.8 | -24.5 | -25.0 | -25.6 | -25.6 |       | -25.5 | -25.4 |       | -26.0 | -25.8 |    |       | -25.8 |       |       | -26.0         | -25.2 | -25.6 |       | 51.31 rice<br>20.0 mungbean |
|                               | -24.7 | -24.1 |       |       | -25.5 |       |       |       |       |       |       |    |       |       | -25.7 |       | -26.3         |       | -25.9 |       |                             |
| rice +<br>mungbean<br>(Vao)   | -24.1 | -24.0 | -24.8 | -24.9 | -24.5 |       | -25.6 |       | -26.0 | -26.4 |       |    | -26.  | -23.4 | -25.7 | -24.8 | -25.5         | -24.9 | -24.9 |       | 22.49 rice<br>8.02 mungbean |
|                               | -24.0 | -24.2 |       |       | -24.3 |       |       |       |       |       |       |    | -23.7 |       | -24.7 | -25.6 | -24.8         |       |       |       |                             |
| rice +<br>fish<br>(Sonia)     | -23.9 | -23.4 | -23.9 | -24.3 | -24.5 |       | -24.7 |       | -24.1 | -24.8 | -23.2 |    |       | -24.7 | -24.7 | -25.1 | -24.9         | -25.2 | -25.2 |       | 15.9 rice<br>4.13 fish      |
|                               | -24.0 | -23.4 |       |       | -24.3 |       |       |       |       |       |       |    |       |       |       |       |               |       |       |       |                             |
| rice +<br>fish<br>(Layda)     | -21.8 | -21.7 | -22.7 | -24.2 | -23.4 |       | -25.4 |       | -26.0 | -26.4 | -26.3 |    |       | -25.9 | -25.8 | -25.9 | -26.3         | -25.1 | -25.5 |       | 34.1 rice<br>2.76 fish      |
|                               | -21.8 | -22.0 | -22.4 |       | -23.9 |       |       |       |       |       |       |    |       |       |       | -26.6 |               |       |       |       |                             |
| rice +<br>milk<br>(Romy)      | -25.0 | -24.4 | -18.6 | -25.6 | -25.4 |       | -26.4 |       | -26.2 | -26.5 | -26.4 |    |       | -26.8 | -25.1 | -24.5 | -25.3         | -25.4 | -25.4 |       | 26.4 rice<br>2.28 milk      |
|                               | -24.9 | -24.3 |       |       |       |       |       |       |       |       |       |    |       |       | -24.4 |       |               |       |       |       |                             |
| rice +<br>milk<br>(Inday)     | -24.6 | -24.7 | -24.9 | -25.5 | -26.1 |       | -26.3 |       | -26.2 | -26.9 | -26.2 |    |       | -26.5 | -26.4 | -27.1 | -26.0         | -26.4 | -26.3 |       | 27.6 rice<br>2.97 milk      |
|                               | -24.5 | -24.4 |       |       | -26.0 |       |       |       |       |       |       |    |       |       |       | -26.8 |               |       |       |       |                             |
| corn +<br>milk<br>(Paula)     | -25.1 |       | -22.1 | -22.2 | -24.1 | -19.7 | -19.0 |       |       |       |       |    |       |       |       |       |               |       |       |       |                             |
|                               | -24.8 |       |       |       | -23.8 | -19.4 | -18.9 |       |       |       |       |    |       |       |       |       |               |       |       |       |                             |

food delta C  
 corn -11.0  
 mungbean -26.0  
 rice -26.8  
 mudfish -22.7  
 milk lipid -30.0  
 milk protein -26.2  
 + lactose

BB = before breakfast  
 AB = after breakfast  
 L = lunch time  
 S = supper time

**Table 2** delta C values of selected body tissues collected from adults consuming the indicated diets.

| Rice (-26.9) - mungbean (-26.1) |                       |                |       |                       |                       |  |
|---------------------------------|-----------------------|----------------|-------|-----------------------|-----------------------|--|
| day #                           | Feces                 | Saliva         | RBC   | blood serum           | urine                 |  |
| 0                               | -24.9                 | -21.1<br>-21.8 | -22.4 | -22.2<br>-22.3        | -18.9                 |  |
| 5                               |                       |                |       |                       | -22.7                 |  |
| 10                              |                       | -23.4<br>-23.4 |       | -23.2                 | -21.9                 |  |
| 15                              |                       | -23.5          |       |                       | -21.5                 |  |
| 20                              | -26.4, -26.1<br>-28.6 | -23.8<br>-23.6 |       | -23.5, -23.2<br>-22.9 | -21.8<br>-27.6, -21.4 |  |

  

| Rice (-26.9) - mudfish (-27.7) |                       |                       |              |                       |                       |                       |
|--------------------------------|-----------------------|-----------------------|--------------|-----------------------|-----------------------|-----------------------|
| day #                          | Feces                 | Saliva                | RBC          | blood serum           | urine                 | breast milk           |
|                                |                       |                       |              |                       |                       | lipid lactose protein |
| 0                              | -24.6, -24.0          | -20.6<br>-20.5        | -26.1, -20.2 | -26.0, -21.8<br>-20.2 | -17.8                 | -27.1 -27.3 -26.8     |
| 5                              |                       |                       |              |                       | -19.1                 |                       |
| 10                             | -27.1                 | -23.1<br>-22.2        |              | -22.6                 | -18.0                 | -27.4 -27.4 -26.5     |
| 15                             | -27.8                 |                       |              |                       | -21.4                 | -26.0 -22.2 -23.8     |
| 20                             | -26.0<br>-27.4, -25.9 | -22.5<br>-22.5, -23.2 |              | -21.3<br>-22.6, -22.9 | -19.3<br>-18.8, -19.0 | -27.3 -25.6 -25.8     |

  

| Rice (-26.9) - milk (lipid - 30.0, lactose + protein - 26.2) |                       |                       |              |                       |                       |  |
|--|-----------------------|-----------------------|--------------|-----------------------|-----------------------|--|
| day #  | Feces                 | Saliva                | RBC          | blood serum           | urine                 |  |
| 0  | -28.6<br>-23.4        | -22.0<br>20.6, -21.1  | -21.5, -25.3 | -22.5<br>-21.2, -21.7 | -22.6, -18.5          |  |
| 5  |                       |                       |              |                       | -20.8                 |  |
| 10   |                       | -24.2<br>-23.4        |              | -22.6                 | -18.9                 |  |
| 15   |                       |                       |              |                       | -19.3                 |  |
| 20   | -26.5<br>-27.0, -28.3 | -27.0<br>-24.2, -23.7 |              | -23.5<br>-22.6, -23.1 | -19.9, -26.1<br>-22.2 |  |

  

| Corn (-11) - mungbean (26.1) |       |        |     |             |       |                       |
|------------------------------|-------|--------|-----|-------------|-------|-----------------------|
| day #                        | Feces | Saliva | RBC | blood serum | urine | breast milk           |
|                              |       |        |     |             |       | lipid lactose protein |
| 0                            | -26.2 | -21.4  |     | -22.3       | -19.3 | -25.5 -22.2 -24.1     |
| 5                            | -15.3 |        |     |             | -15.5 | -18.8 -13.8 -12.0     |
| 10                           | -14.8 | -16.9  |     | -20.3       | -14.9 | -19.3 -12.7 -12.8     |
| 15                           | -14.2 |        |     |             | -13.2 | -19.3 -12.2 -16.5     |
| 20                           | -15.5 | -16.9  |     | -19.1       | -14.8 | -18.1 -15.9 -16.1     |

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Table 3 delta N values of selected body tissues collected from adults consuming the indicated diets.

Rice (+4.0) - mungbean (-1.0)

| <u>day #</u> | <u>Feces</u> | <u>Saliva</u> | <u>serum</u> | <u>urine</u> |
|--------------|--------------|---------------|--------------|--------------|
| 0            |              | +7.5          | +9.1         | +13.8        |
| 5            |              |               |              | 6.3          |
| 10           |              | 6.9           | 8.6          | 7.8          |
| 15           |              |               |              | 8.2          |
| 20           | +4.3         | 7.1           | 7.8          | 7.2          |

Rice (+4.0) - fish (+13.0)

| <u>day #</u> | <u>Feces</u> | <u>Saliva</u> | <u>serum</u> | <u>urine</u> | <u>breast milk protein</u> |
|--------------|--------------|---------------|--------------|--------------|----------------------------|
| 0            |              | +6.1          | +9.3         | +13.0        | +6.6                       |
| 5            |              |               |              | 11.6         |                            |
| 10           |              | 8.6           | 9.6          | 10.8         | 10.7                       |
| 15           |              |               |              | 9.5          | 10.9                       |
| 20           |              | 9.7           | 10.2         | 12.2         | 10.9                       |

Rice (+4.0) - milk (+4.5)

| <u>day #</u> | <u>Feces</u> | <u>Saliva</u> | <u>serum</u> | <u>urine</u> |
|--------------|--------------|---------------|--------------|--------------|
| 0            |              | +7.9          | +9.1         | +9.8         |
| 5            |              |               |              | 9.7          |
| 10           |              | 8.1           | 9.2          | 14.4         |
| 15           |              |               |              | 12.6         |
| 20           | +7.7         | 8.8           | 8.9          | 12.7         |

Corn (+3) - mungbean (-1.0)

| <u>day #</u> | <u>Feces</u> | <u>Saliva</u> | <u>serum</u> | <u>urine</u> | <u>breast milk protein</u> |
|--------------|--------------|---------------|--------------|--------------|----------------------------|
| 0            |              | +8.2          | +10.6        | +8.6         | +9.4                       |
| 5            |              |               |              | 4.7          | 9.7                        |
| 10           |              | 7.1           | 9.3          | 3.9          | 8.0                        |
| 15           |              |               |              | 9.5          | 3.4                        |
| 20           |              | 6.7           | 8.6          | 1.8          | 8.1                        |

Figure 1. delta C values of breath carbon dioxide collected from adults consuming the indicated diets for twenty consecutive days.

