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

Final Report May 1989

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).

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SUMMARY

The stable isotopes of carbon, nitrogen and hydrogen, when used as they occur naturally, were found to be of mixed value as indigenous tracers of nutrient assimilation by humans. The variety of foods consumed by the test subjects, even under the conditions of the controlled dietary intakes provided during this research, and the mix between the use of body tissues and recently ingested foods for metabolic processes reduces the usefulness of these natural isotopes to qualitative indicators of short-term, in-vivo nutrient flow. This is in sharp contrast to the usefulness of the isotopes of carbon for tracing nutrient assimilation in animals. In cases where an animal is confined and provided with a controlled diet, the isotopes of carbon provide a quantitatively exact measure of the fractional assimilatiion of diet components.

In studies of human or animal nutrition, when 13C or 15N are added to the diet in large excess of the amounts naturally found in foods, both 13C and 15N have been shown to be exact, quantitative tracers of nutrient assimilation.

In this research, which is oriented to human nutrition, the isotopes of carbon (referred to as delta C) were found to be the most useful of the three isotope pairs. Feces delta C approached the value of the ingested foods within five days of the start of a diet. The delta C value of exhaled carbon dioxide reflected a mixture of sources (current foods plus body store) used for metabolic energy. Saliva and blood serum delta C values were usually similar. In the course of a diet constant for 20 days, the delta C of these fluids indicated a metabolically active amino acid pool taken 30% from the current diet and 70% from catabolized body tissues. The most rapid indicator of the current diet was breast milk lactose. Within 12 hours of a dietary change, the lactose delta C value approached the delta C value of the dietary carbohydrate. Changes in breast milk protein were somewhat less rapid. Breast milk lipid required approximately five days to approach the diet delta C.

Delta N values of the body sources tested (feces, saliva, urine, serum, breast milk) show a statistically significant correlation with the foods ingested during the previous ten days. The standard estimate of error is, however, large (two to three delta N units, or 2 o/oo to 3 o/oo) relative to the range of delta N values usually present in available foods (10 units, or 10 o/oo). This large uncertainty results in part from the strong fractionation of nitrogen within the body. Tissues have delta N values 1 o/oo to 6 o/oo (averaging 3 o/oo to 4 o/oo) more positive than the diet from which they are formed.

Delta D values of body tissues showed little or no consistent trend with dietary input. Even when our subjects consumed constant diets for periods of 20 days, we found slight trend of delta D for the body sources sampled relative to the delta D of the diet.

The most significant finding of this research is the demonstration that, based on delta C data, breast milk lactose and to

a large extent breast milk protein is formed from the immediate diet, and more specifically from the carbohydrate of that diet. In attempting to influence rates of production of milk, special attention must be given to this dietary component since its influence extends far beyond the provision of energy to the lactating mother.

OVERALL PROJECT OBJECTIVES

The objectives of this project were 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. With this information, we were then to attempt to measure the assimilation (or lack of assimilation) of several dietary components typically used in the area of the Philippines where the research was conducted (Cabuyao, Laguna; some 30 km from the Los Banos campus of the University of the Philippines, UPLB).

Evaluation of the usefulness of these isotope pairs as natural tracers was accomplished. The findings were not affirmative in all cases. This required considerable repetition to ensure that the lack of correlations which we found was valid. We had planned to maintain adults on single component diets for 45 consecutive days. This we found to be impractical. The subjects, although eager to be part of the experiment, experienced discomfort after two or three weeks. We were able to maintain adults on single component diets for 20 consecutive days. Our several attempts to maintain young children on confined diets did not meet with success. As soon as bowel movements became irregular or other discomfort became apparent, the parents (understandably) insisted that the diet be terminated. We therefore directed our efforts toward nursing mothers. Even here, if the infant experienced loose bowel movements, the parents (the husband exercises considerable influence on the activities of his nursing wife) terminated the experiment. This was several times the case when the mother was consuming milk as her main protein input, even though the diet was a balanced one from the aspect of calories and carbohydrate:protein:fat.

We succeeded in a significant, though not primary objective in this research. The major part of the project was performed in the Philippines, and not in a main city of the Philippines. The assimilation of the technology was sufficient for the cooperating investigator, Dr. Nieva Librojo of UPLB, to join in a second CDR project.

PROJECT REPORT

Introduction and Background

In regions of the world where malnutrition is rampant, one or two foods often supply in excess of 75% of the total caloric and protein intake. When a diet has only slight variety, one or two tracers can, in theory, provide the information needed to evaluate the relative assimilation of these few dietary components. The stable isotopes of carbon, nitrogen and/or hydrogen, as they occur naturally in the consumed foods, might provide this information.

For convenience, the following standard convention has been adopted for the reporting of data related to these isotope pairs:

$$\text{delta C} = \left(\frac{(^{13}C/^{12}C)\ \text{sample}}{(^{13}C/^{12}C)\ \text{standard}} - 1\right) \times 1000\ \text{(per mil)}.$$

A corresponding transformation is used for delta N and delta D. The standards are PDB carbonate for carbon, atmospheric nitrogen for nitrogen and Standard Mean Ocean Water (SMOW) for hydrogen. This has been discussed in detail in several publications (c.f., DeNiro and Epstein, 1978; Schroeder, 1983).

The theoretical benefits of these tracers is that they are naturally occurring and as such already exist as measurably different values in normally consumed foods. No added tracers may be necessary.

They are not radioactive and so do not produce any exceptional hazard in their use. They are therefore applicable for situations as sensitive as nursing mothers.

In plants, delta C values fall into two main categories, referred to as C-3 type and C-4 type. The categories originate from the manner in which the plants fix carbon from atmospheric carbon dioxide into their plant tissues. C-3 type plants have delta C values in the range of -25 o/oo to -32 o/oo. C-4 type plants have delta C values in the range of -9 o/oo to -14 o/oo (Table 1). A given plant species has a given delta C value throughout the world, provided it is grown in the open atmosphere.

Delta N values of a plant are dependent upon the delta N value of the available nitrogen in the soil, just as the delta C values of a plant are dependent upon the delta C of the carbon dioxide of the ambient air. Because this delta N varies with the type of fertilization, the delta N value of a given plant species can vary considerably even within a given community (Table 1).

Delta D values of a plant are dependent upon the delta D of the water used by the plant. This can vary within short geographic distances and so plant type is not an indicator of the delta D of the plant (Table 1). In general as distance from the equator increases, the delta D of rain water becomes more negative (that is it contains relatively less deuterium). The water molecule, HDO, weighs a bit more than HHO. Globally, most evaporation occurs in the tropics, and so most cloud vapor is formed there. As these vapors move to increasing latitudes, there is a slight perference for the heavier molecules to rain out. The result is that the remaining water vapor is lighter, or has a more negative delta D value.

It is these naturally occurring differences in delta values that give the potential for isotope pairs to be tracers in nutrition of humans in a manner similar to that used in studies of lower animal nutrition. For example if a person consumes as the main portion of the diet two foods, one C-3 and one C-4, the relative assimilation of these foods may be reflected in the delta C values of newly formed tissue, excrement and breath carbon dioxide. Since in effect "you are what you eat," the entire isotopic composition of a body might be expected to reflect the values of the diet. This has been found to be the case for a variety of animals, including humans, provided the diet averages more or less constant delta values for long periods of times (at least many months) or over greater than 5-fold weight gains (Schoeller et al., 1986; our work reported here, Figures 1 and 2).

Technical Work Plan

To evaluate the fidelity of the isotope pairs as tracers in human nutrition, free living subjects were provided with controlled diets for periods ranging up to 20 consecutive days. The original plan was to maintain the diets for up to 45 days. The discomfort experienced by the test subjects on such strict diets made 20 days the maximum period tested. The diets consisted of a single component (either a C-3 or C-4 type plant) or of a mix of two foods, at times one C-3 and one C-4, and at times both C-3 or both C-4. In all cases vitamins and minerals and water were supplied. For variety, one small fruit or vegetable was permitted daily. Samples of newly formed body sources were collected at pre-determined intervals during the trials. These sources consisted of feces, urine, breath, saliva, blood serum, facial shavings, and breast milk separated, by centrifuging, into its

fractions of lactose, lipid and protein. The single component diet provided a base which indicated how closely the body would match the diet. The 2-component diet allowed a balance of protein and carbohydrate to be consumed by the subjects and so resembled more closely normal dietary intake.

Groups of six to 12 volunteers were selected for each feeding trial. Although the research in the Philippines was based at the Los Banos campus of the University of the Philippines (UPLB), the trials were performed in a village, Cabuyao, Laguna. This village is located some 30 km from UPLB. The volunteers included male and female adults, lactating mothers and their nursing children. All volunteers were examined by a trained physician to ensure their fitness for the program. Because the trials were conducted in a village and the volunteers all lived locally, meals were able to be provided three times daily at a single location. Usually samples were collected at the time of the meals. Subjects were allowed to take some foods home for between-meal snacks.

Samples were refrigerated until being dried at 90 C. The dried samples were ground to uniformity. For each sample, a five to 15 mg portion was put into a clean 6 mm diameter, 1 mm wall thickness, 15 to 20 cm long borosilicate glass (such as Pyrex brand) tube which had been flame sealed at one end. One hundred to 150 mg copper oxide (made from fine copper powder heated for 1 hour at 500 C) and 2 to 5 cm of 5 micron diameter silver wire (such as BDH product number 11022) were added to the tube. The open end of the tube was attached to a vacuum line and, while at vacuum, the tube was flame sealed. If the intent for a particular sample was only to measure delta C, then the tube might be sealed at room pressure (the amount of carbon

contained in the air's carbon dioxide within the small volume of the tube is miniscule compared to the carbon in the five to 15 mg of sample). The sealed samples were combusted at 500 C for at least 16 hours. The resulting gases were analysed for deltas C, N and/or D at one of three commercial isotope separation mass spectometer laboratories.

Agreement among the three laboratories for duplicate samples was excellent for delta C and delta N, being better than 0.5 o/oo for both. Unfortunately the same can not be said for delta D. Here values for replicate samples varied by as much as 60 o/oo for samples having an average delta D value of 100 o/oo. This made comparison of delta D data among laboratories not possible. Since there was a consistency within each laboratory, we decided to work with one lab, having then at least the possibility of looking at relative changes even if we were uncertain of the absolute value of delta D.

The diets presented to the subjects consisted of local staples. In this way the experiment simulated as closely as possible the usual diets of the community. The dietary regimes were as follows: corn only; rice only; corn plus mungbean; rice plus mungbean; rice plus fish; rice plus milk; corn plus milk.

Results and Discussion

Delta C (Tables 1, 2, 4, 5; Figures 1, 3 - 7)

Delta C has proven itself to be the most useful tracer of the three isotope pairs tested in this research. It has, however, quite significant limitations when the diet being tested has departed from the food usually consumed by the test subject during the months prior to the experiment. When such changes are involved, the body source

used for evaluation must be carefully selected.

We (work reported herein) and others (Schoeller et al 1986) have found that over long periods in which the delta C, delta N and delta D of the diet have been maintained at a fairly constant average, the isotope values of blood, hair, feces come to match these values. This constancy is the usual case for stable populations of a given locality. Thus we found less than 2 o/oo variation in delta C values in hair taken from local residents of Wuxi, China, a city inland from Shanghai. There hair delta C averaged -22 o/oo. Animal feeds sampled in this area had values that averaged at -22 o/oo. The milk from Wuxi had a delta C of -21.2. This average value for hair at Wuxi was quite different from another city in the Far East, Bangkok, where the delta C of residents' hair was -18 o/oo. The Bangkok value reflects the high intake of seafoods (delta C approximately -16 to -19) by residents of this city. Hair taken from residents of Chicago (work by Schoeller, op. cit.) and of Pennsylvania (our study) had delta C values all between -15.4 and -17.7. Within two families (five members or more per family), the range was less than 1 o/oo. The only exception was in one family. There, the one family member who maintained a diet in which neither meat nor fish were consumed had a value 2 o/oo different from that family's average. The similarity of delta C values in these two separated locations in the United States is reflected in the similarity in the delta C of foods. Powdered milk was sampled in the USA for its delta C values. For milk taken from California, Florida and Pennsylvania, the delta C values ranged only from -18.0 to -19.7. i.e., a spread of less than 2 o/oo. Animal feeds are apparently similar in these locations. This close agreement in the delta C of powdered milk was the same within all countries

tested, although the absolute values differed greatly among the countries: USA -19 (skim); Israel -22 (28% fat); Denmark -26 (28% fat); Australia and New Zealand -29 (28 % fat); Australia -26 (skim). Though sampled less intensively than was hair, the blood serum delta C data also had less than a 2 o/oo spread over all samples taken within these stable populations.

The delta C is constant in these cases because stable populations tend to eat a diet that provides on the average, a constant source of isotope intake.

A goal of our research was to determine the fidelity of delta C as an indicator of relatively short term changes in diet. It is in such cases that the specific body source must be considered. The data included in the accompanying tables and figures are indicative of the results attained in this study.

In cases where the subject is having normal bowel movements (at least one per day), within five days the stool delta C matched the dietary intake of carbohydrate in cases when oats or rice were the carbohydrate source. This was true for rice-only diets as well as for diets in which the rice (delta C -27) was supplemented with a protein-rich food such as fish (delta C -23), mungbean (delta C -26) or milk (delta C -28). When corn was the source of carbohydrate, regardless of whether consumed in a corn-only diet or when the corn was supplemented by a protein-rich food such as milk (delta C -21) or mungbean (delta C -26), the stool delta C was consistently more negative than the corn, having a final value of between -15.5 and -16.6. This discrepancy between fecal delta C and diet delta C for diets in which corn was the main carbohydrate may in part be

attributed to the lignin content of the corn. Processed white rice and processed oats (as used in these diets) are low in lignin. The grain is therefore largely digestible. In the corn there is a partition between the lignin and the starch plus protein fractions. This concentrates lignin in the stool. By separating corn into its fiber and non-fiber fractions, we found that corn lignin is 5 o/oo to 7 o/oo more negative than the total corn grain. Total corn solids are 1 o/oo to 3 o/oo more negative than the corn solubles. These observations can account for much of the shift in fecal delta C relative to the dietary corn delta C and the lack of such a shift in the rice-based diets.

The importance of these findings is that even when measuring stool delta C values as an indication of diet digestibility, one must consider the partition that may occur within the individual components of the diet. Processed rice or oats are recommended for such studies since partition has not been found.

Urine delta C correlated with the delta C of the main protein food, being 4 ± 2 o/oo less negative than the protein assimilated. This was true for rice-only diets (-27 for the rice; -22 for the urine) and for more balanced diets. Thus when rice (-27) and fish (-23) were consumed, the urine had a delta C of -19. When rice (-27) and milk (-26) were consumed, the urine delta C was -21. When Israeli milk (-21) was substituted in this diet, the urine delta C shifted to -17. As with feces, when corn was the main dietary carbohydrate, the delta C of urine did not follow the rice-related trend. Both for corn-only diets (delta C -11) and for diets in which corn was consumed with a protein-rich food such as mungbean (delta C -26) or milk (-21), the urine delta C consistently stabilized at a value

which fell between -15 and -18 o/oo. This value range encompasses the delta C values of feces in the corn-related diets. We do not have an explanation as to why the delta C of urine and feces should be so similar in corn-related diets, when in rice plus protein food diets, the urine and feces had distinctly unequal delta C values.

Neither saliva nor blood serum delta C values were satisfactory tracers of short term changes in the diet. With hindsight, it was possible to account for the observed trends by attributing the sources of these amino acid-rich body fluids to an active amino acid pool being composed of 30% recently assimilated foods and 70% to catabolized body tissues. While the delta C values conform to this 3:7 distribution, the resulting delta C value is so dependent upon the body store, that small variations in this value mask much larger variations in the relative assimilation rates of the current dietary components.

In the rice-related diets, with a balance of carbohydrate and proteins, delta C values of breath carbon dioxide matched those of the diet provided that the diet was kept constant for at least three days. When corn only was consumed, the breath delta C showed a clear measure of body tissues being catabolized, although this catabolism was not at the same rate in each subject tested. With the corn-only diet, the morning breath delta C (after the all-night fast) was 2 to 3 o/oo more negative (closer to the stable, pre-diet value) than the breath delta C five hours after breakfast had been consumed (figures 6A and 6B). When the corn was supplemented with milk, the difference between morning and noon breath delta C was usually 1 o/oo or less. The morning-noon changes in breath delta C were a few tenths of a

o/oo in the rice-based diets. Since the stable breath values (i.e., the pre-diet values) were about -24 o/oo, switching to rice, rice-bean or rice-milk represented a change of only - 2 to -3 o/oo in diet delta C, whereas switching to corn-only represented a change in diet of +13 o/oo, and switching to corn-bean or corn-milk represented a change of +6 o/oo. Hence, shifts between catabolism as in the fasting, pre-breakfast, samples and the noon samples where the day's food is contributing to the energy pool, will produce a more extreme change in breath delta C with the corn-based diets than with the rice-based diets. Breath delta C appears to be a valuable tracer for current sources of metabolic energy.

Of all the body sources tested, breast milk was found to be the most rapidly influenced by the immediate diet. Delta C of the breast milk lactose came to within 4 o/oo of the dietary carbohydrate within 12 hours of a change from C-3 to C-4 and from C-4 to C-3 type carbohydrates. This change continued during periods up to 20 days of sampling. Breast milk protein and lipid changed more gradually, reaching this equilibrium after two to five days of a new diet. For diets of rice plus fish, corn plus mungbean and corn plus fish, the breast milk lactose delta C was similar to the breast milk protein delta C and both were similar to the dietary carbohydrate. The lipid fraction was consistently 2 o/oo to 6 o/oo more negative than the lactose and protein. This more negative value for the lipid is found in all plant and animal tissues.

Delta N (Tables 1, 3, 4; Figures 2, 3, 5, 7)

The relationship between the delta N of assimilated foods and that of body tissue forming from this food is less exact than that of delta C. With animals confined to a rigid dietary regime, the

relation between food and tissue delta C values has a standard estimate of error of less than 1 o/oo over a 20 o/oo range in delta C values (figure 1). With similarly confined animals, the standard estimate of error in delta N is 2.2 o/oo over a delta N range of only 10 o/oo (figure 2). The spead of 4.4 in the uncertainty of prediction encompasses over 40% of the potential values of delta N. Thus although the correlation between delta N of tissue and delta N of food is valid (probability of chance being < 0.0001), the correlation coefficient (r^2) is only 0.6, as compared with >0.9 in the diet-body tissue relationships for delta C.

Delta N data related to long term diets are presented in figure 2. These data include those generated in this research as well as those of DeNiro and Epstein (1981). It is worth noting that the data of DeNiro and Epstein, by themselves, also show only a weak correlation between diet and body delta N values.

For animals consuming the same diet for long periods of time (and for stable populations of humans, as discussed in relation to delta C, above), the average delta C of newly formed protein tissue is 0 to 1 o/oo less negative than the utilized food. That is the tissue closely matches the food. The average delta N of newly formed tissue is 3 o/oo to 4 o/oo more positive than the food. This large excursion in tissue delta N from the delta N of the food is due to fractionation of amine groups during metabolic transamination. The extra neutron in 15N, as compared to 14N, in the small amine groups makes a the significant weight difference in the molecule and so affects rates of diffusion and chemical reaction.

All of the body sources tested in this research reflected the

weak relation between delta N of recently consumed foods and delta N of body tissue. In all cases the tissue was more positive than the diet delta N, however the difference between tissue and diet ranged, with no apparent pattern, from 0.5 o/oo to 10 o/oo more positive. This held true for protein-poor diets (rice only or corn only) and for balanced diets (rice plus fish; rice plus milk; corn plus mungbean).

A survey of the delta N data listed in table 3 demonstrates this poor match between diet and body delta N. After 20 days of constant diets, with average delta N values ranging from +1 o/oo to +8 o/oo, the delta N values in saliva, blood serum and breast milk protein ranged from +7 to +11. Urine samples showed a wider range, but no clear or consistent relationship to the consumed diet delta N value.

Considering this inexactness in delta N, in cases other than those in which diets have been constant for very long times, or during which 5-fold or more weight gains have been achieved, delta N does not appear to be a consistently reliable, quantitative tracer. This not the case when large amounts of 15N are artificially added to a diet. In such cases, 15N (or delta N) has proven to be a reliable tracer (cf., the extensive work of CNRC at Baylor College of Medicine, Houston, Texas).

Delta D (Tables 1, 4; Figures 8A, 8B, 8C)

The usefulness of the stable isotopes of hydrogen, when used in their natural concentrations, as tracers in the food web is more problamatic than that of nitrogen. Results of delta D analyses were found to be sensitive to techniques used, as was discussed in the Technical Work Plan section. The lack of a clear trend in the delta D data for the data taken in this research is shown in table 4 and

figure 8. Schoeller et al (ibid) found a match between average delta D of ingested foods and delta D of blood plasma and hair. Our work with confined animals did not show any trend between delta D of supplied feed and delta D of animal muscle.

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Table 1 Isotopic values and related data for foods used in this project.

C4 foods

Food	delta C	delta N	delta D
corn	-11.4		-16
corn	-11.4		-10
corn	-11.2	+16.5	+ 8
corn	-11.2	+16.5	-14
corn	-11.2	+11.4	-12
corn	-10.2	+10.2	-31
corn	-12.1		-26
corn	-11.8	+ 2.0	-28
corn	-11.3	+ 2.7	-10
corn	-10.9	+ 1.9	-18
corn	-11.4	+ 1.9	-61
corn	-11.2		-58
corn	-10.5	-1.1	- 9
Kellogg's	-12.0	+16.5	+ 8
corn-		+11.4	-14
flakes		+11.4	-12
cane sugar	-11.3		- 0
cane sugar	-11.4		+12
cane sugar	-11.4		+ 8
cane sugar	-10.8		-34
cane sugar	-10.9		-16
cane sugar	-11.0		- 4
pineapple	-11.6		- 3
pineapple	-13.1		
average ± S.D.			

Foods of animal origin

	delta C	delta N
Australian full fat milk	-28.7 to -29.3	+3.3 to +3.7
Australian skim milk	-26.2	+3.8 to +5.1
Danish full fat milk	-25.9	
USA skim powder milk	-18.0 to -19.7	
Israeli milk	-21.6 to -23.3	
Israeli eggs	-15.8 to -17.9	+ 4.6
Israeli chicken	-17.8	
Phil. mudfish	-22.7	+13.5
N.Atl. cod	-18.7	
whitefish	-17.8	
salmon	-17.5	
halibut	-15.9 to -17.7	

Table 1 continued

C3 foods

Food	delta C	delta N	delta D
peanut	-26.3		
peanut	-26.3		
beet sugar	-25.6		
beet sugar	-25.6		
beet sugar	-25.7		
rice	-26.3		-44
rice	-27.5	+4.8	-55
rice	-25.3	+5.6	-48
rice	-26.9	+3.3	-45
rice	-27.4		-54
rice	-26.7		-38
rice	-26.0		-50
coconut meat	-25.7		
coconut meat	-26.9		-163
coconut meat	-25.9		-156
coconut oil	-27.5		-164
coconut oil	-27.0		-178
oats	-26.9	+5.3	-63
oats	-26.9	+8.8	-61
oats	-26.6	+6.9	-60
oats	-26.6	+7.6	-59
Cheerios	-26.2		
wheat	-25.3		
wheat	-25.4		
soy	-25.0		
tofu	-28.7	+0.3	
soy protein	-24.6	+0.1	
avocado	-28.8		
perumin	-28.9		
tomato	-29.1		
banana	-24.8		
onion	-25.0		
guava	-25.8		
guava	-27.7		-60
cabbage	-27.0		
mungbean	-25.8	+2.7	-83
mungbean	-25.9	-1.5	
dates	-23.8		
dates	-24.3		
chayote	-26.2		

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	-22.4	-22.2	-18.9
		-21.8		-22.3	
5					-22.7
10		-23.4		-23.2	-21.9
		-23.4			
15		-23.5			-21.5
20	-26.4, -26.1	-23.8		-23.5, -23.2	-21.8
	-28.6	-23.6		-22.9	-22.6, -21.4

Rice (-26.9) - mudfish (-22.7)

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

Rice (-26.9) - milk (lipid - 30.0, lactose + protein - 26.2)

day #	Feces	Saliva	RBC	blood serum	urine
0	-28.6	-22.0	-21.5, -25.3	-22.5	-22.6, -18.5
	-23.4	-20.6, -21.1		-21.2, -21.7	
5					-20.8
10		-24.2		-22.6	-18.9
		-23.4			
15					-19.3
20	-26.5	-27.0		-23.5	-19.9, -26.1
	-27.0, -28.3	-24.2, -23.7		-22.6, -23.1	-22.2

Corn (-11) - mungbean (-26.1)

day #	Feces	Saliva	RBC	blood serum	urine	breast milk lipid	breast milk lactose	breast milk 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

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

Rice (+4.0) - mungbean (-1.0)

day #	Feces	Saliva	serum	urine
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)

day #	Feces	Saliva	serum	urine	breast milk protein
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)

day #	Feces	Saliva	serum	urine
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)

day #	Feces	Saliva	serum	urine	breast milk protein
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

n1

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	delta D
boiled corn	-10.5	-1.1	-9
boiled rice	-27.5	+4.8	-55

		corn diet			rice diet		
	day	delta C	delta N	delta D	delta C	delta N	delta D
saliva	0	-21.8	+10.2	-102	-22.5	+9.5	-81
	16	-18.0	+9.3	-97	-23.5	+9.3	-101
	20	-16.2	+9.8	-96	-23.2	+9.2	-100
urine	0	-22.0	+7.3	-59	-21.7	+6.9	-47
	16	-16.9	+6.1		-22.5	+7.1	-52
	20	-17.7	+5.2	-87	-22.8	+5.5	-60
feces	0	-25.8	+6.8	-125	-24.7	+6.0	-120
	16	-16.9	+4.3	-98	-26.6	+6.3	-126
	20	-15.6	+3.4	-100	-26.6	+5.1	-142
hair	0	-20.2	+10.0	-72	-20.8	+9.5	-69
	16	-17.6	+9.3	-105	-21.4	+9.5	-58

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

Table 5 delta C of carbon dioxide in breath collected from subjects consuming the indicated diets.

day #	0		1		2		5			10				15			19		20	kg food eaten
	BB	AB	L	S	BB	S	BB	AB	BL	BB	AB	BL	S	BB	L	BS	BB	L	BB	
corn (Pandy)	-23.4 -23.7		-21.8	-21.6	-23.3		-21.7 -21.7		-19.4	-20.9 -20.8		-19.0		-20.2 -20.3	-17.6		-18.3	-16.5	-18.4 -18.5	38.1 corn
corn (Loreng)	-23.2 -23.6		-22.8				-21.7			-22.4 -22.7				-21.5					-21.9	38.4 corn
corn + mungbean (Lito)	-23.9 -24.4	-21.8 -23.0	-19.2	-21.3	-22.3		-20.4 -20.8		-18.0	-19.9 -20.3		-19.0		-19.2	-19.5 -18.4			-14.9	-18.7 -19.2	40.9 corn 11.8 mungbean
corn + mungbean (Leyda)	-23.2 -22.6	-22.8	-20.0				-15.2	-15.9		-16.8	-16.9			-17.5	-17.6		-14.9		-14.9 -14.2	34.8 corn 22.1 mungbean
rice + mungbean (Lily)	-24.8 -24.7	-24.5 -24.1	-25.0	-25.6	-25.6 -25.5		-25.5	-25.4		-26.0	-25.8			-25.8	-25.7		-26.0 -26.3	-25.2	-25.6 -25.9	31.3 rice 20.0 mungbean
rice + mungbean (Veo)	-24.1 -24.0	-24.0 -24.2	-24.8	-24.9	-24.5 -24.3		-25.6		-26.0	-26.4			-26.4	-23.4 -23.7		-25.7	-24.8 -24.7	-25.5 -25.6	-24.9 -24.8	22.5 rice 8.02 mungbean
Gener	rice + mungbean						-25.2		-25.8					-25.4	-25.8		-25.7	-25.6		19.7 rice 14.7 mungbean
rice + fish (Sonia)	-23.9 -24.0	-23.4 -23.4	-23.9	-24.3	-24.5 -24.3		-24.7		-24.1	-24.8		-23.2		-24.7		-24.7	-25.1 -24.9	-24.9	-25.2 -25.2	15.9 rice 4.13 fish
rice + fish (Leyda)	-21.8 -21.8	-21.7 -22.0	-22.7 -22.4	-24.2	-23.4 -23.9		-25.4		-26.0	-26.4		-26.3		-25.9	-25.8		-25.9 -26.6	-26.3	-25.1 -25.5	34.1 rice 2.76 fish
rice + milk (Romy)	-25.0 -24.9	-24.4 -24.3	-18.6	-25.6	-25.4		-26.4		-26.2	-26.5		-26.4		-26.8	-25.1		-24.5 -24.4	-25.3	-25.9 -25.9	26.4 rice 2.28 milk
rice + milk (Inday)	-24.6 -24.5	-24.7 -24.4	-24.9	-25.5	-26.1 -26.0		-26.3		-26.2	-26.9		-26.2		-26.5	-26.4		-27.1 -26.8	-26.0	-26.4 -26.3	27.6 rice 2.97 milk
corn+ milk (Paula)	-25.1 -24.8		-22.1	-22.2	-24.1 -23.8	-19.7 -19.4	-19.0 -18.9													
corn+milk (Virgie)							-20.2							-19.5					-20.4	41.0 corn 3.00 milk
(Veo) corn+milk	-23.1		-21.4	-20.9	-18.3		-23.7		-22.5	-23.9		-23.0		-24.0	-20.8		-23.6	-22.3	-24.0	28.3 corn 2.79 milk

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

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

Figure 1. delta C values of muscle tissue taken from animals consuming a single-component diet during a period in which these animals exerienced a greater than 5-fold weight gain vs the delta C of the diet.

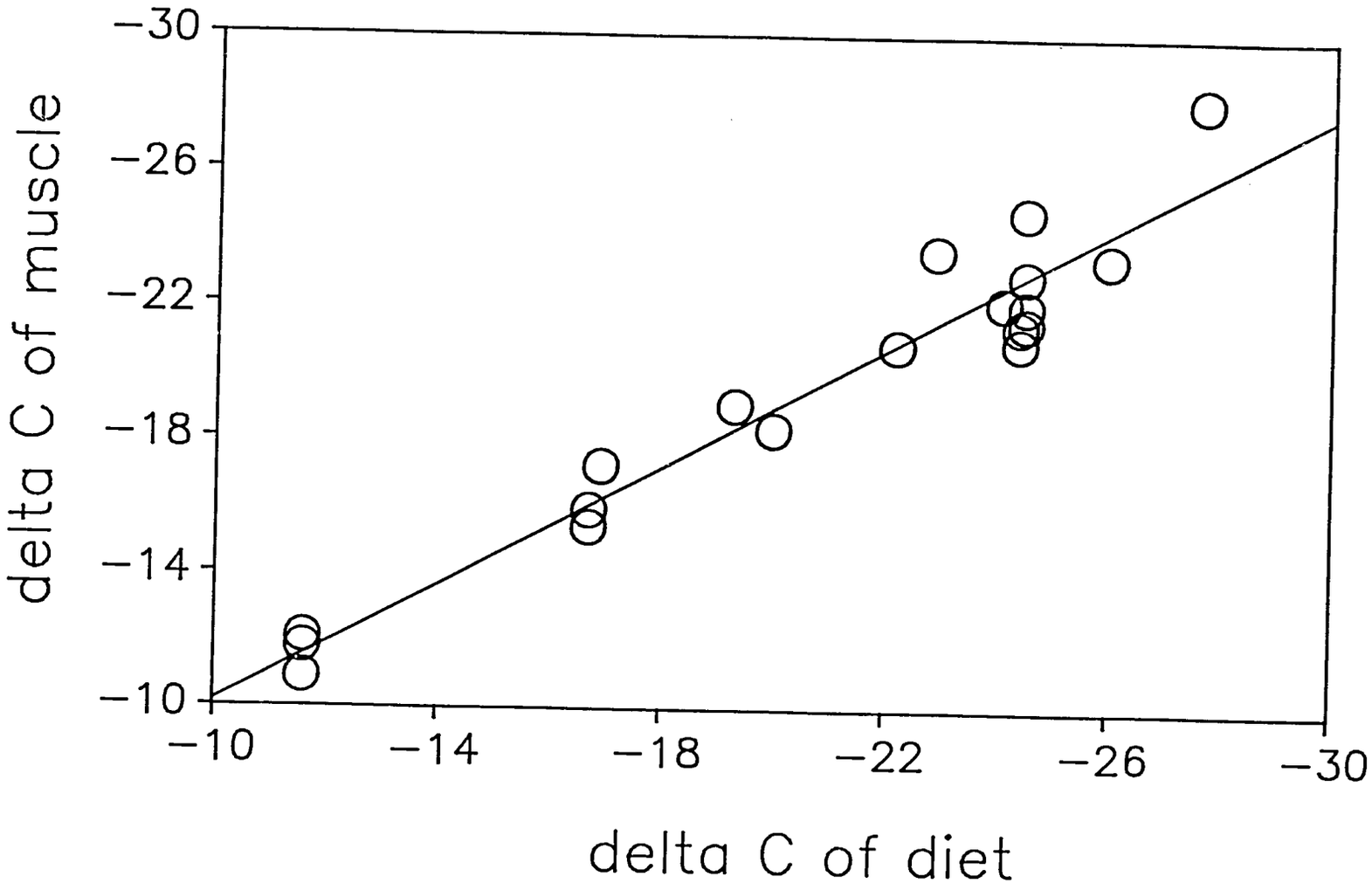


Figure 2. delta N values of muscle tissue taken from animals consuming a single-component diet during a period in which these animals experienced a greater than 5-fold weight gain vs the delta N values of the diet.

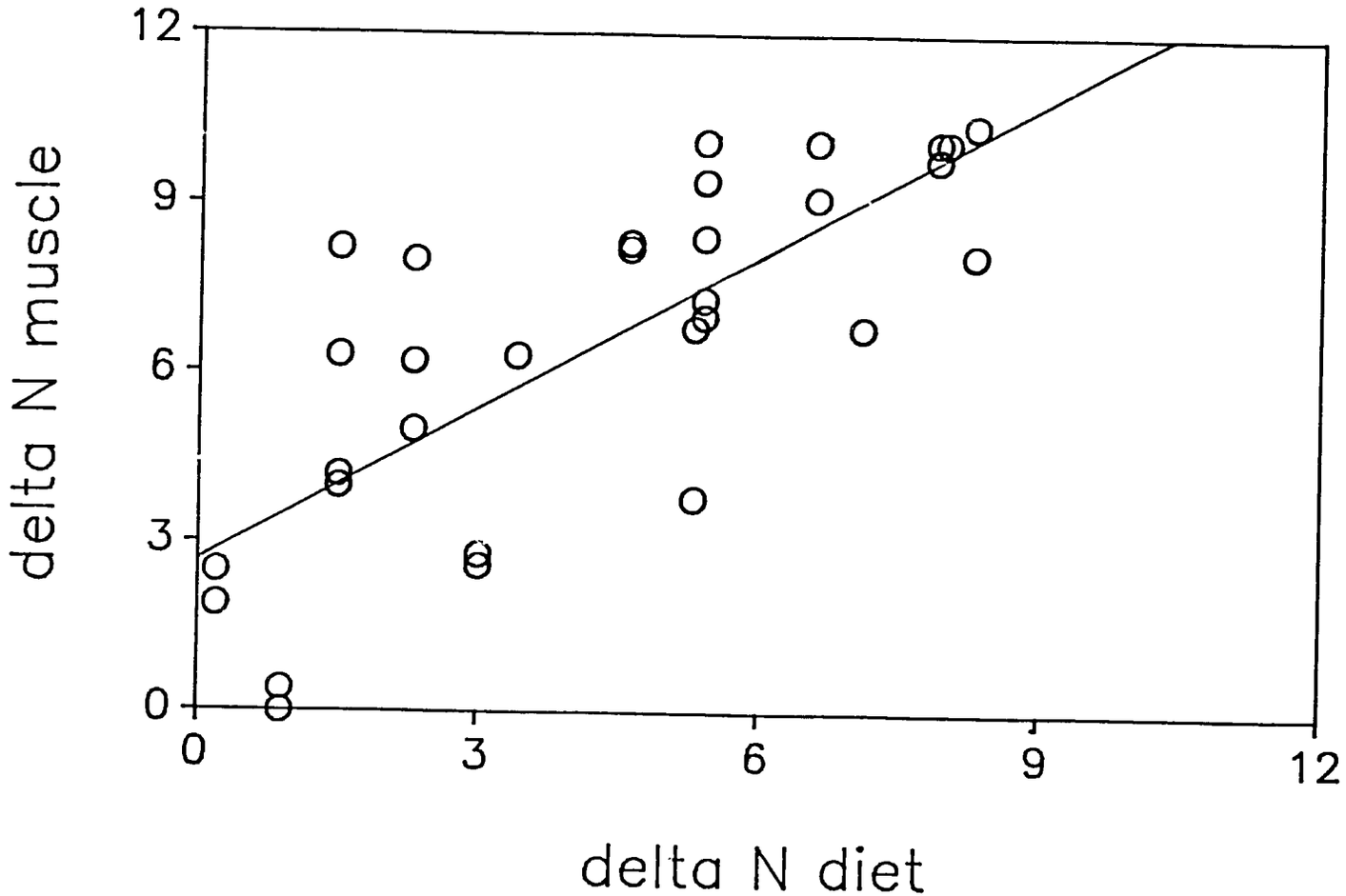


Figure 3A. delta C and delta N values of selected body tissues including breath and breast milk fractions (lipid, lactose, protein) taken from a nursing mother consuming a corn (delta C -11.0, delta N +3) plus mungbean (delta C -26.1, delta N -1) d for twenty consecutive days.

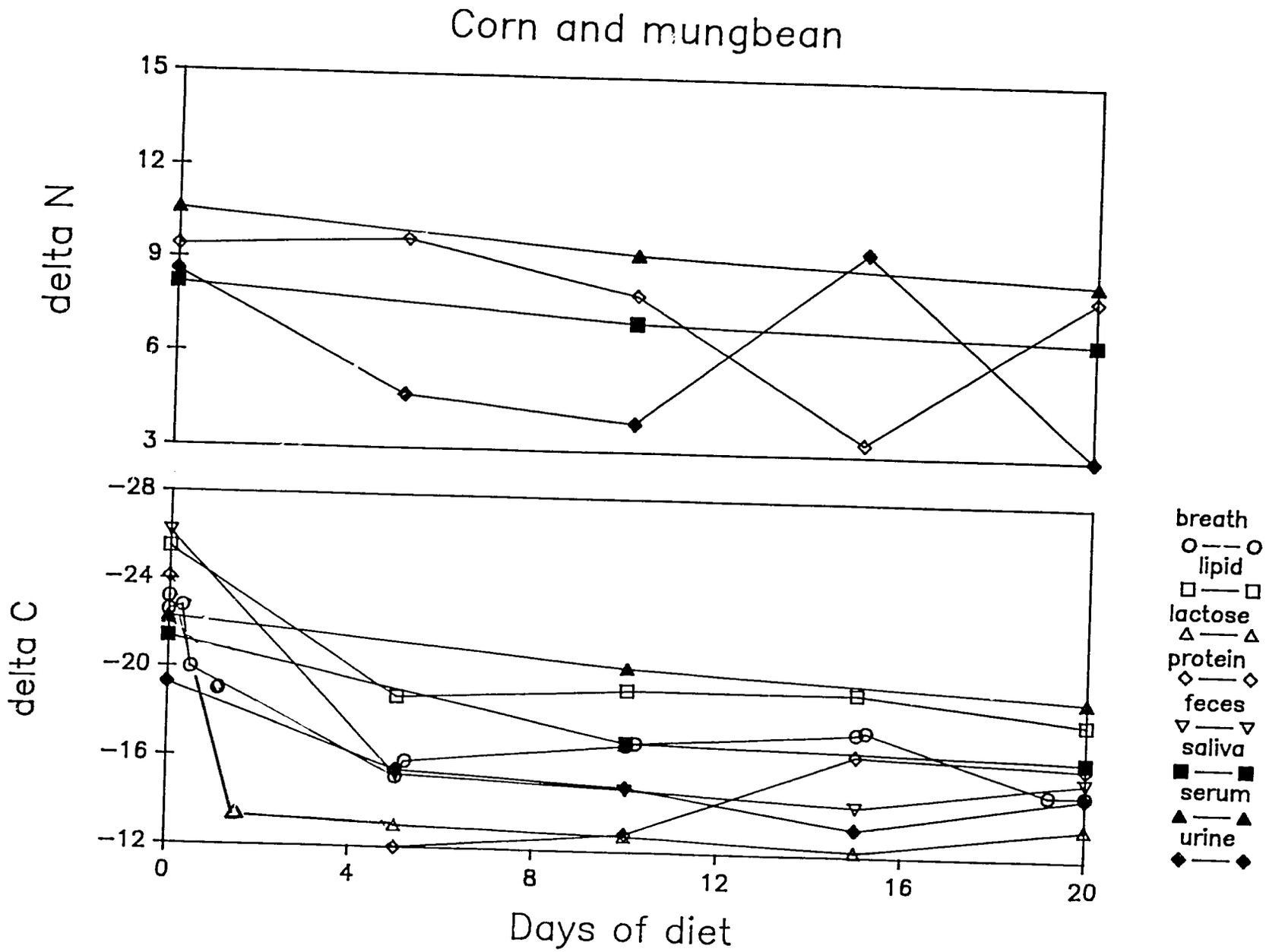


Figure 3B. delta C and delta N values of selected body tissues including breath and breast milk fractions (lipid, lactose, protein) taken from a nursing mother consuming a rice (delta C -26.9, delta N +4) plus mudfish (delta C -22.7, delta N +13) diet for twenty consecutive days.

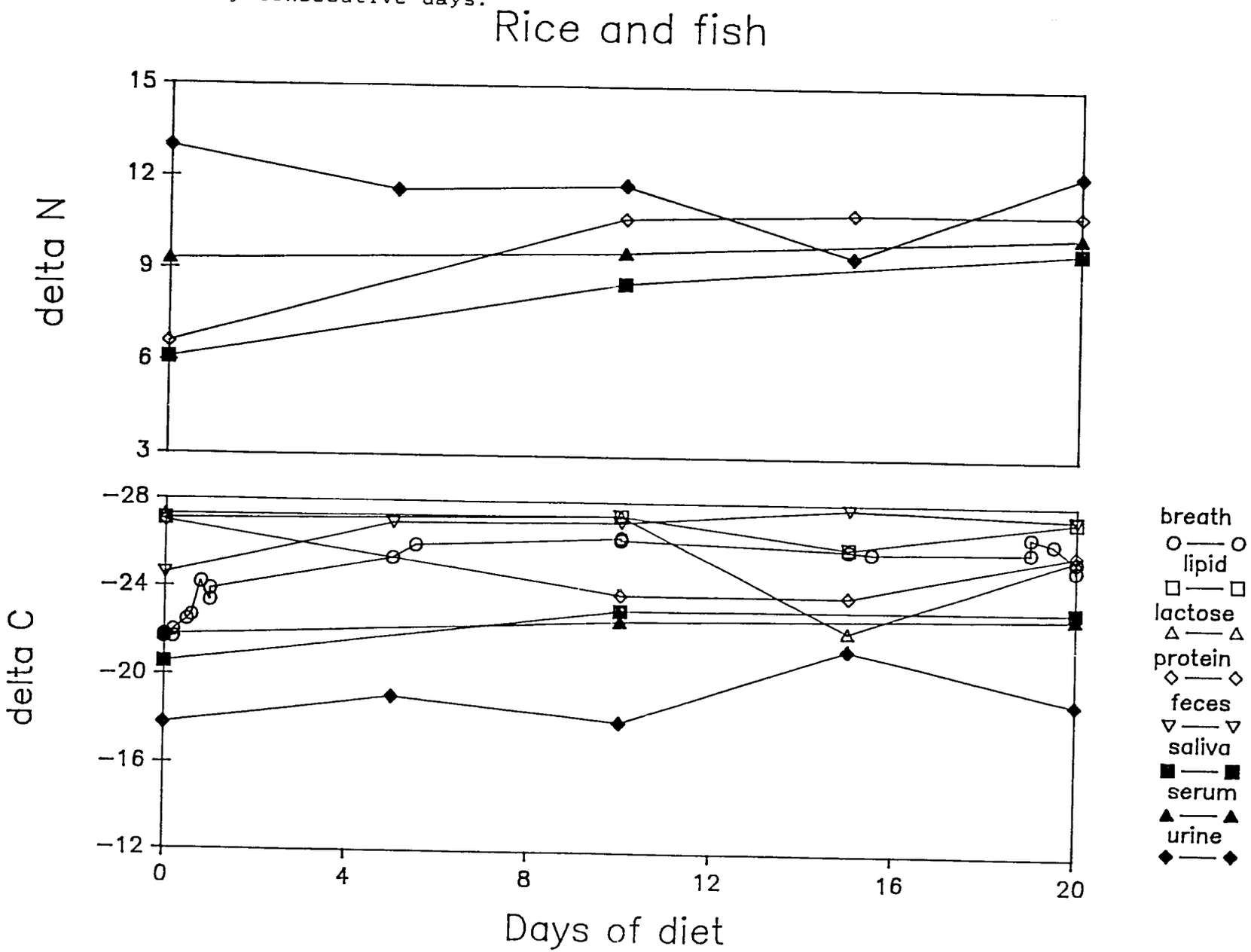
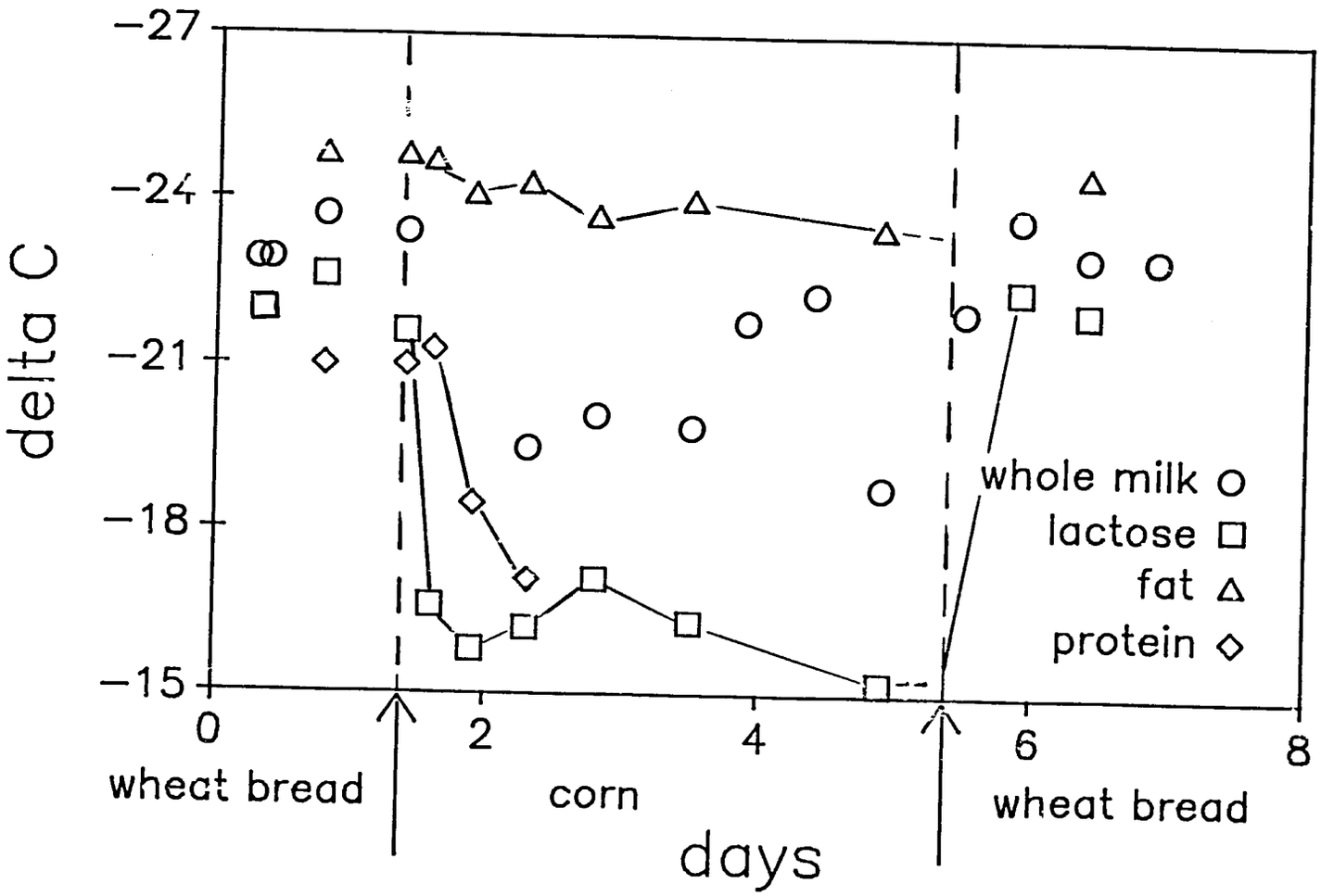
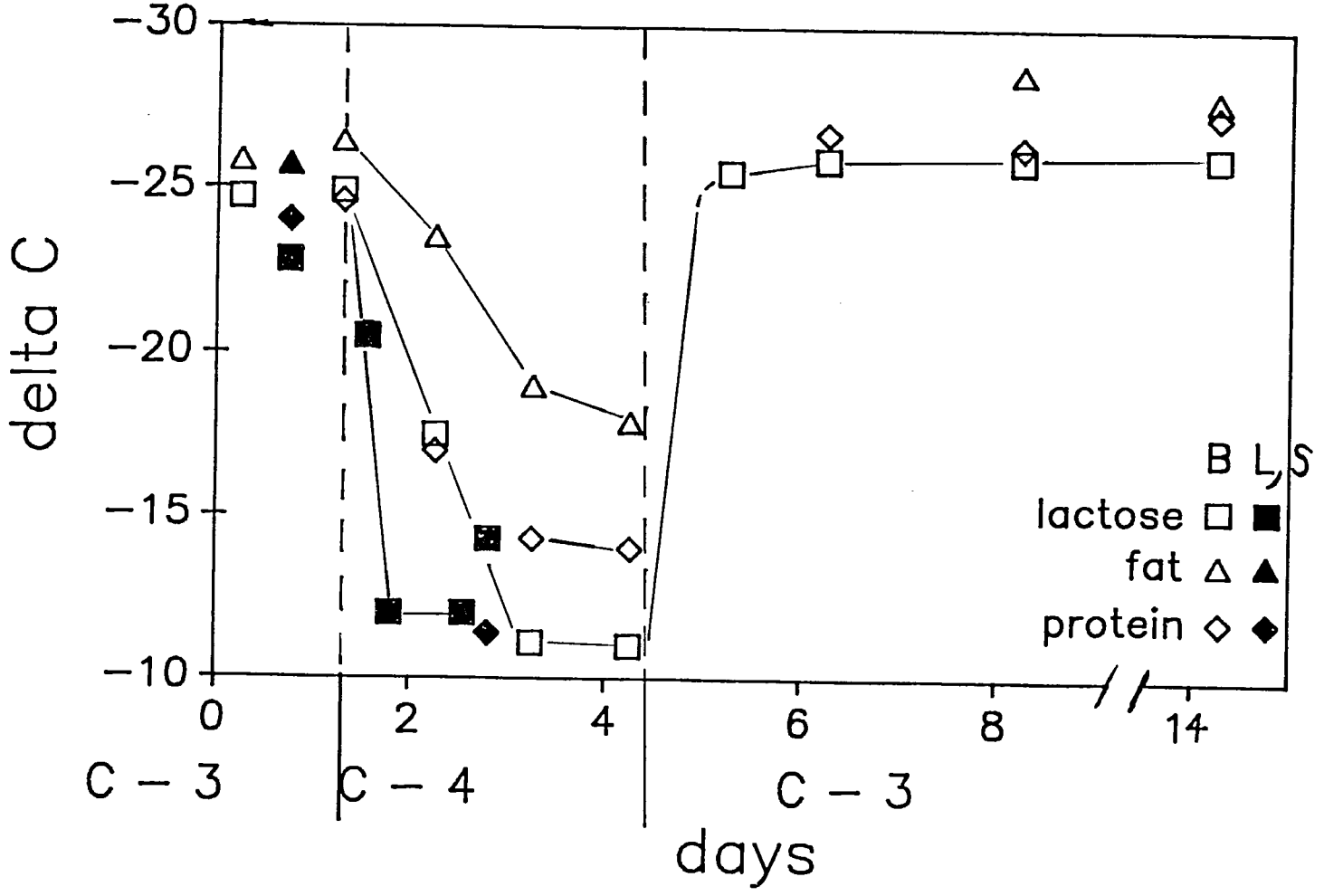


Figure 4. delta C values of breast milk fractions sampled from a mother nursing twice daily during a diet change from C-3 (delta C -25) to C-4 (delta C-11) and then to C-3 foods.
4A: Only the grain products were changed while continuing consumption of dairy (delta C -23) and vegetable (delta C -25) foods throughout the entire experimental period.
4B: The entire diet was either C-3 or C-4 during the indicated periods.

4A



4B


-30
-25
-20
-15
-10
delta C
0
2
4
6
8
14
C - 3
C - 4
C - 3
days
B L,S
lactose
fat
protein

C-3

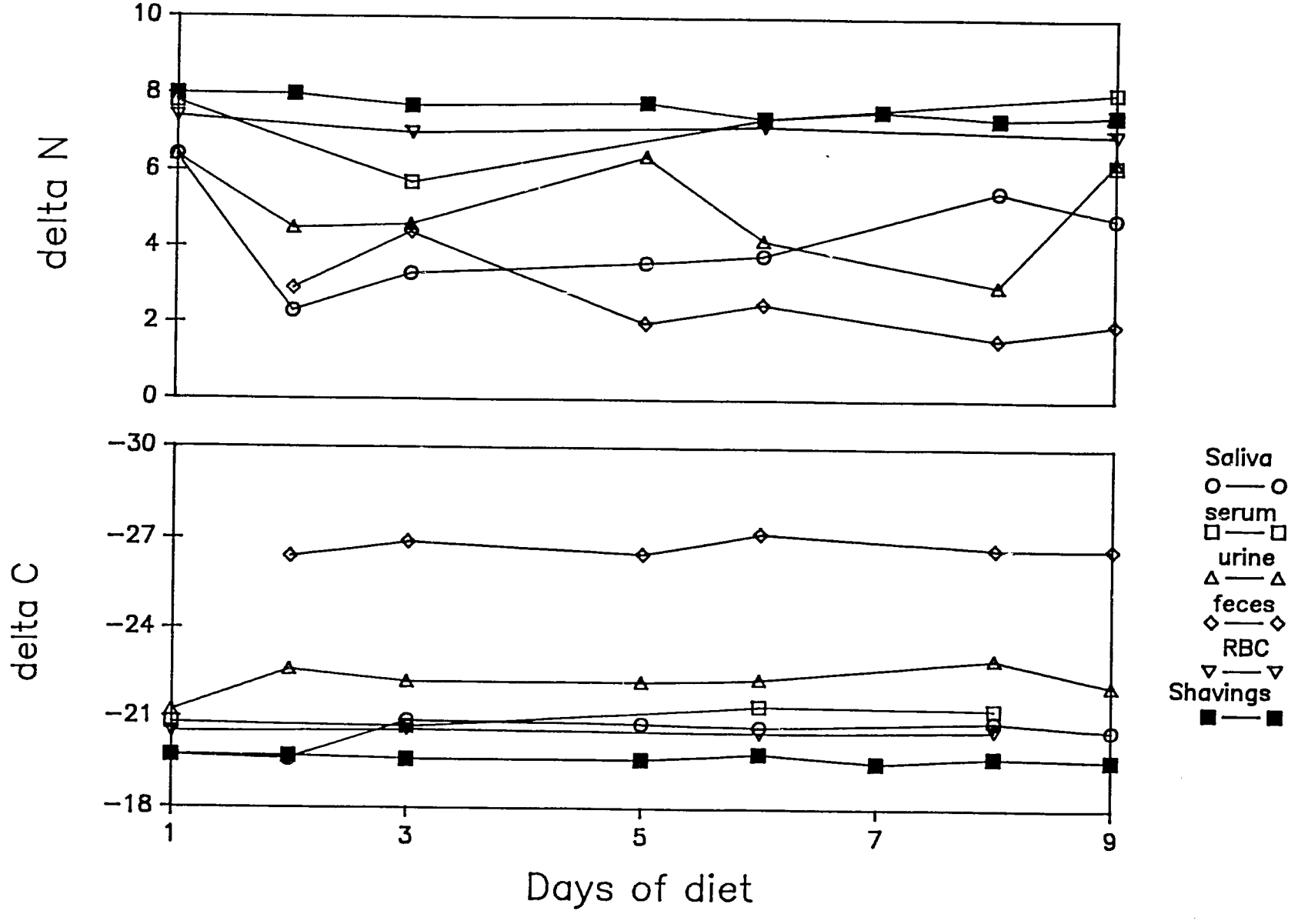
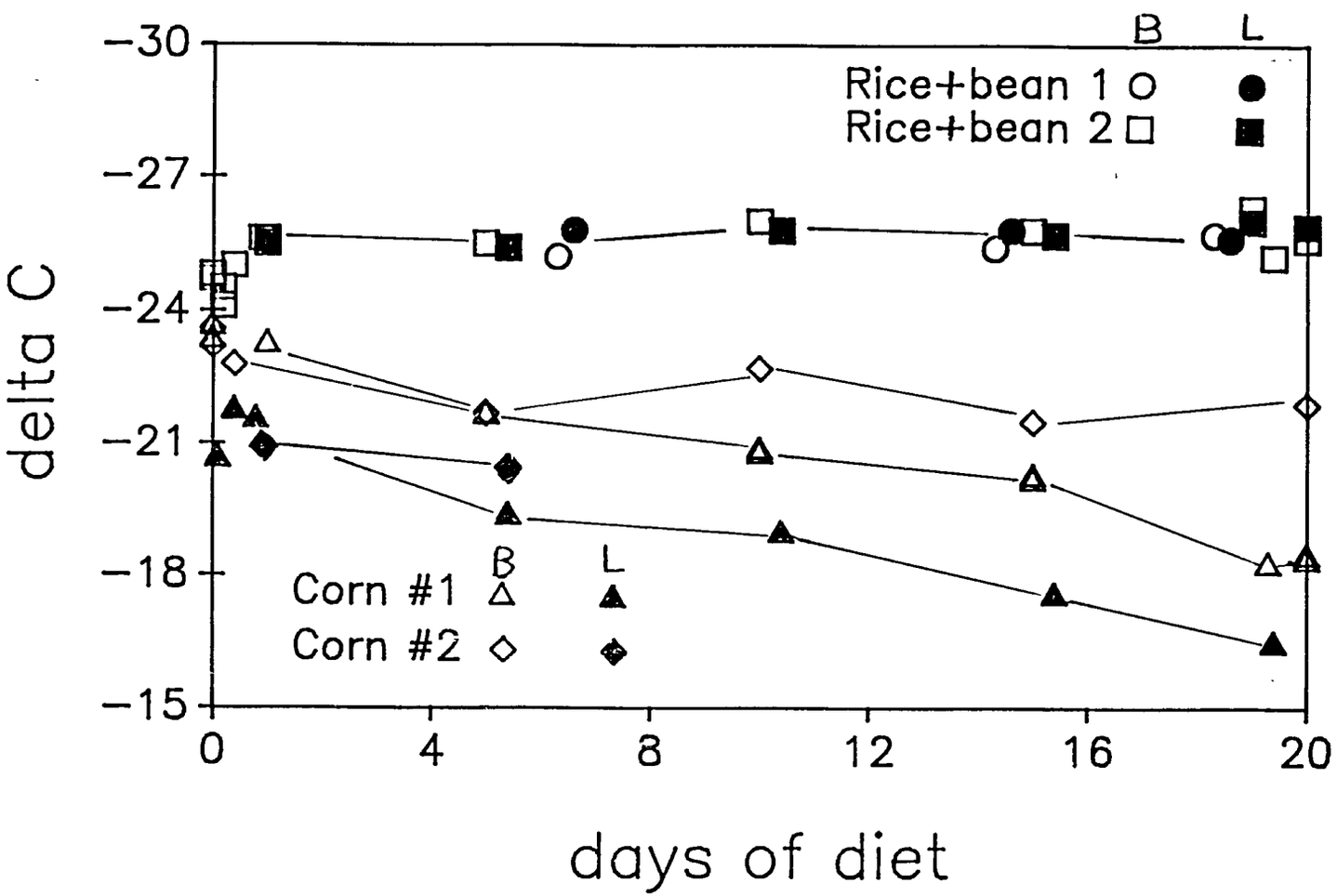


Figure 5. delta C and delta N values of selected body tissues collected from an adult male consuming only C-3 foods (delta C -25 to -29) for nine days. Animal protein during this period was from 28% fat powdered Australian milk (delta C -28.7), with a protein plus lactose delta C -26.2, delta N +3.5. For thirty days prior to this period all animal protein consumed had delta C values of -21 to -23.

Figure 6. delta C values of breath carbon dioxide collected from adults consuming the indicated diets for twenty consecutive days. (delta C values: rice -26.9; mungbean -26.1; corn -11.2; milk -28.7, having a lactose plus protein delta C -26.2).
(B sambles collected before breakfast; L samples collected at lunchtime).

6A



6B

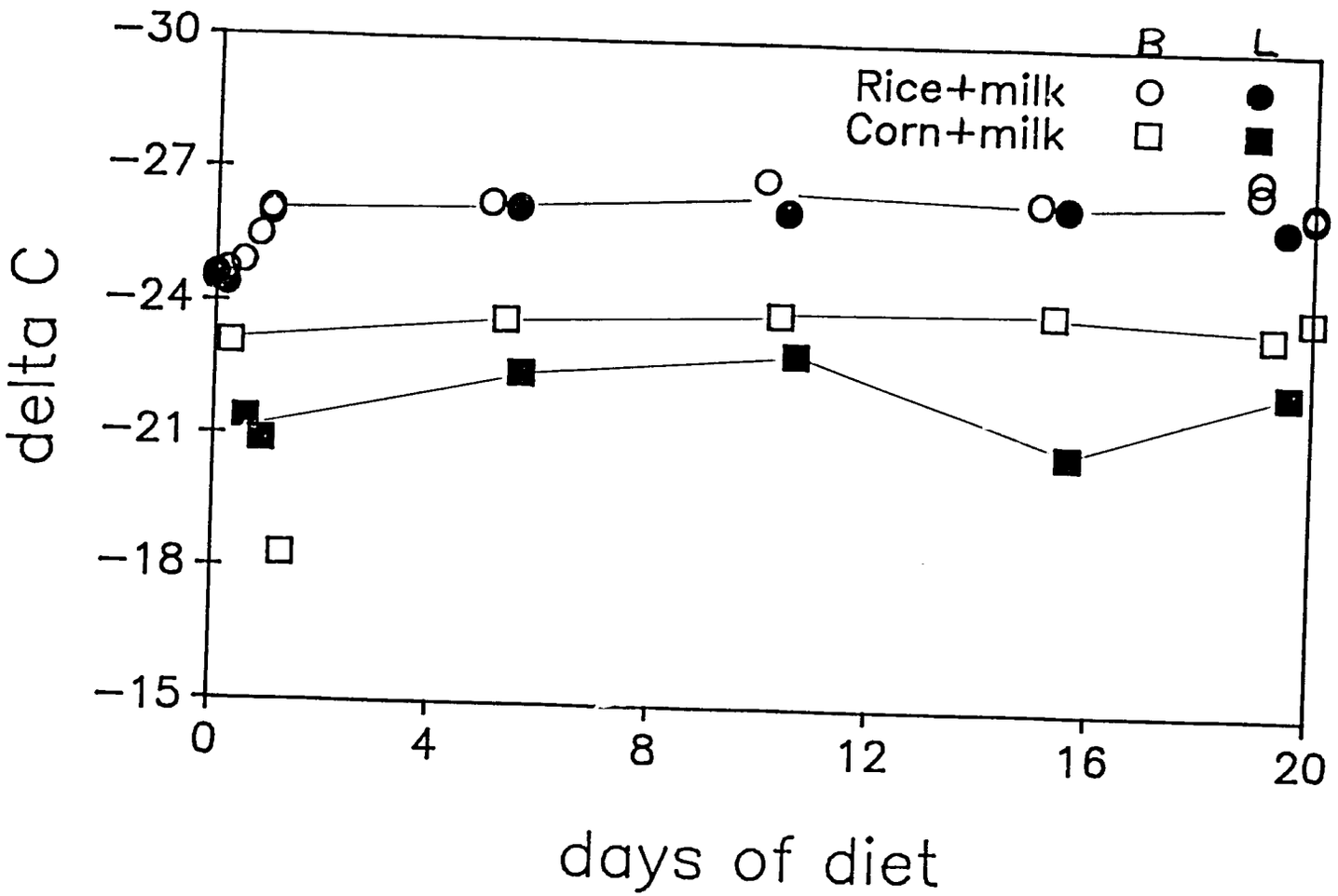

B
L
Rice+milk
Corn+milk
−30
−27
−24
−21
−18
−15
delta C
0
4
8
12
16
20
days of diet

Figure 7. delta C and delta N values of selected body tissues collected from adults consuming soy protein (delta C -24.6, delta N +0.3) and oats (delta C -26.7, delta N +7) for ten consecutive days followed by a two day period of mixed C-3 and C-4 type foods.

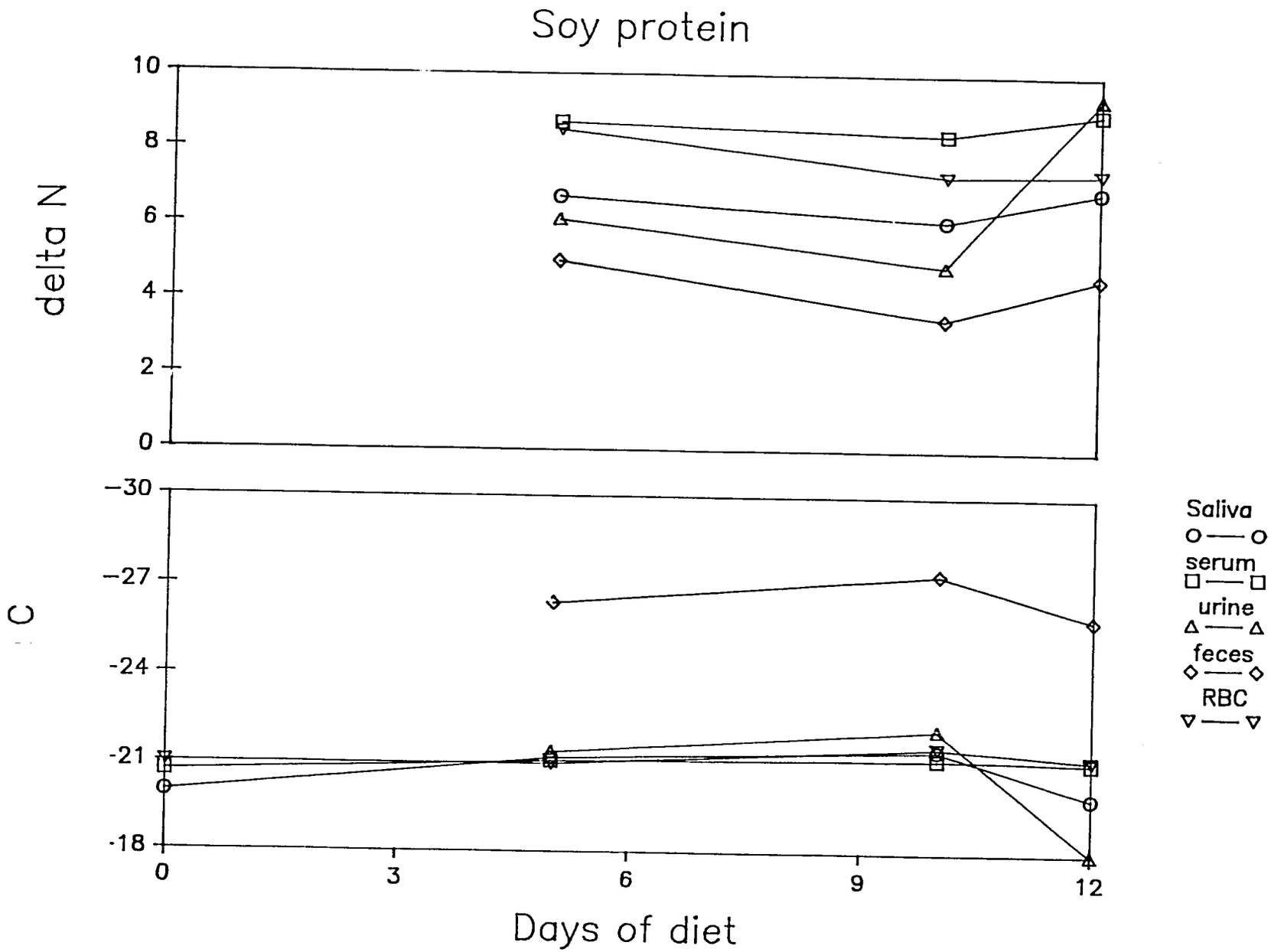
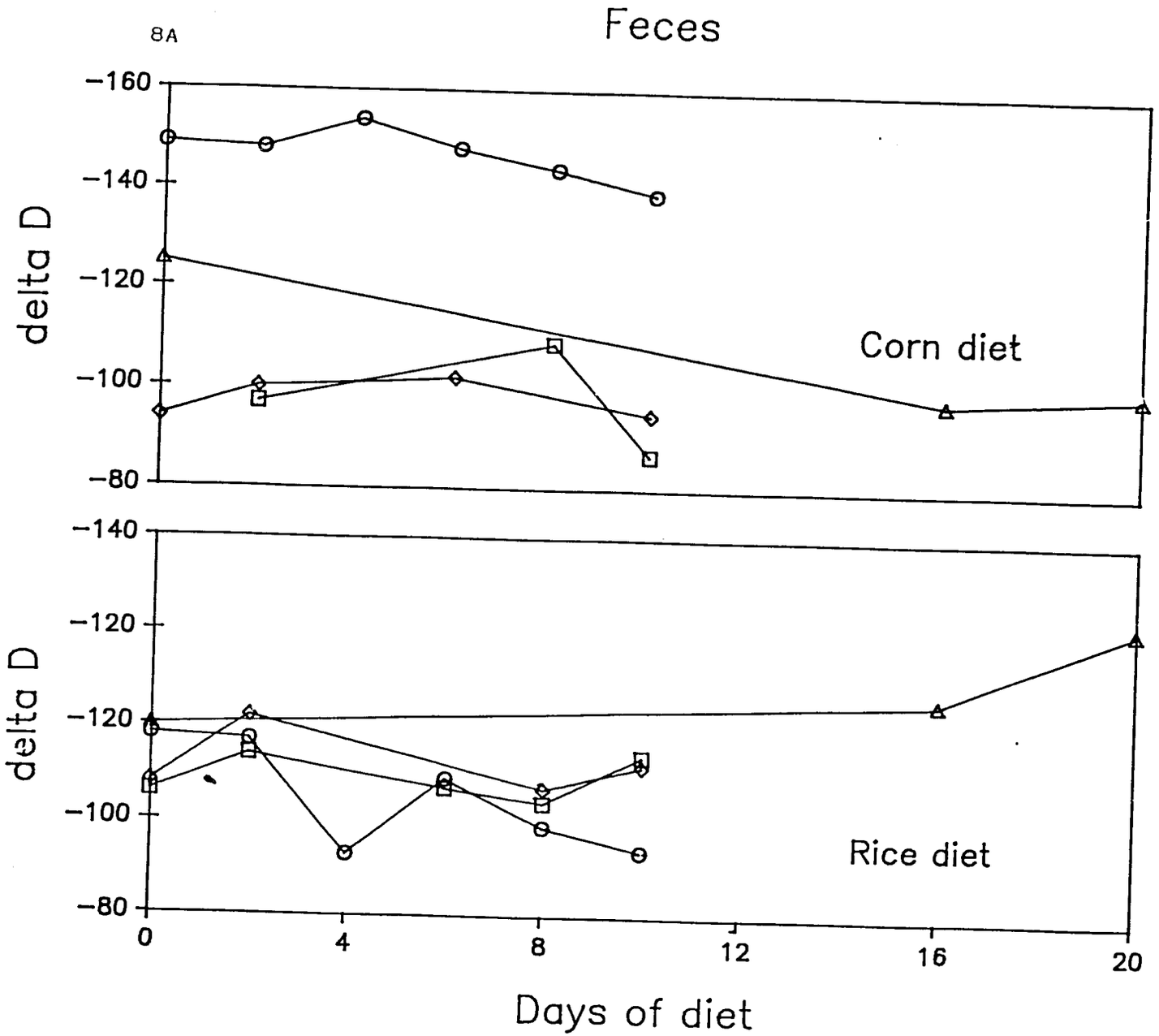
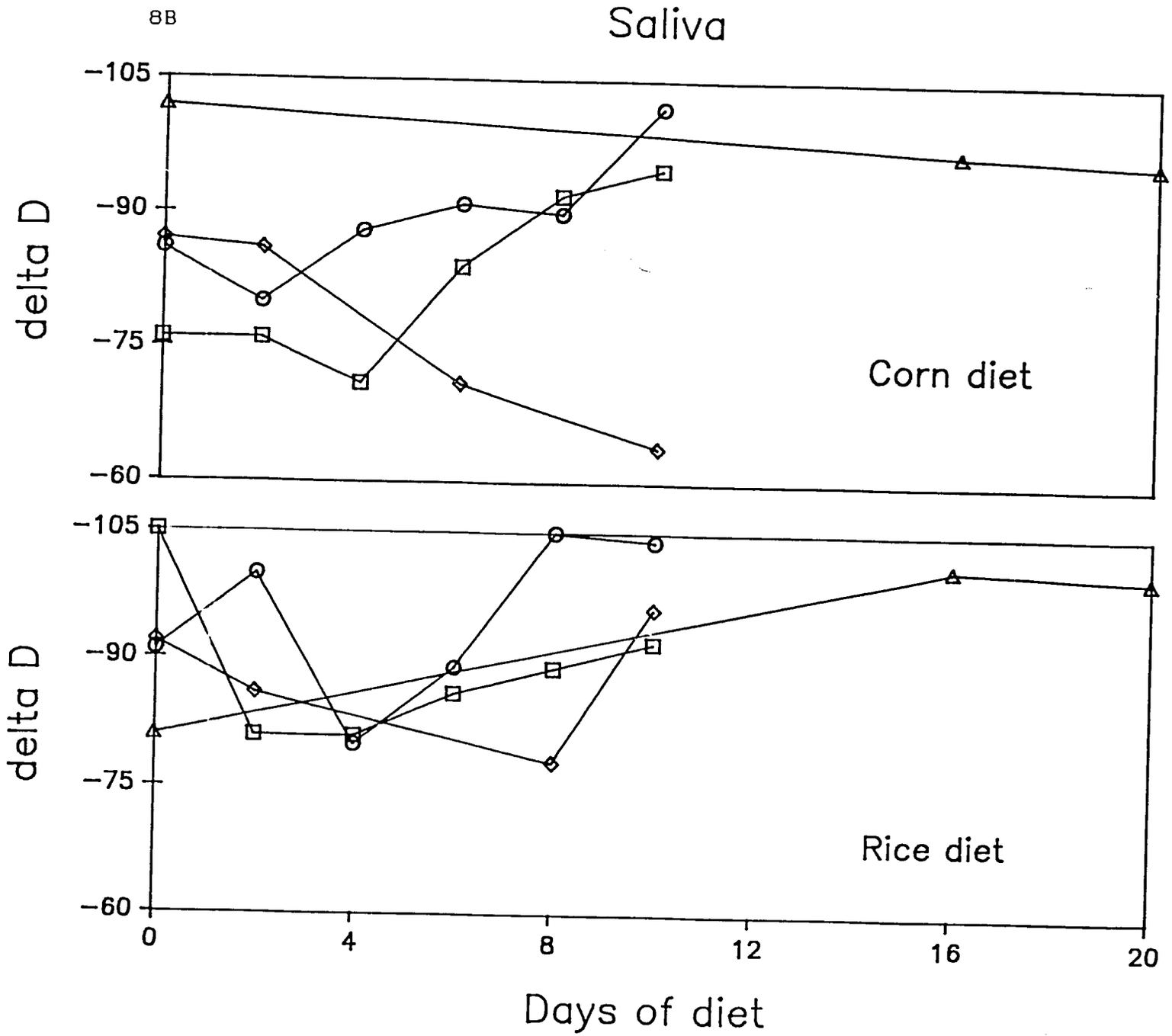


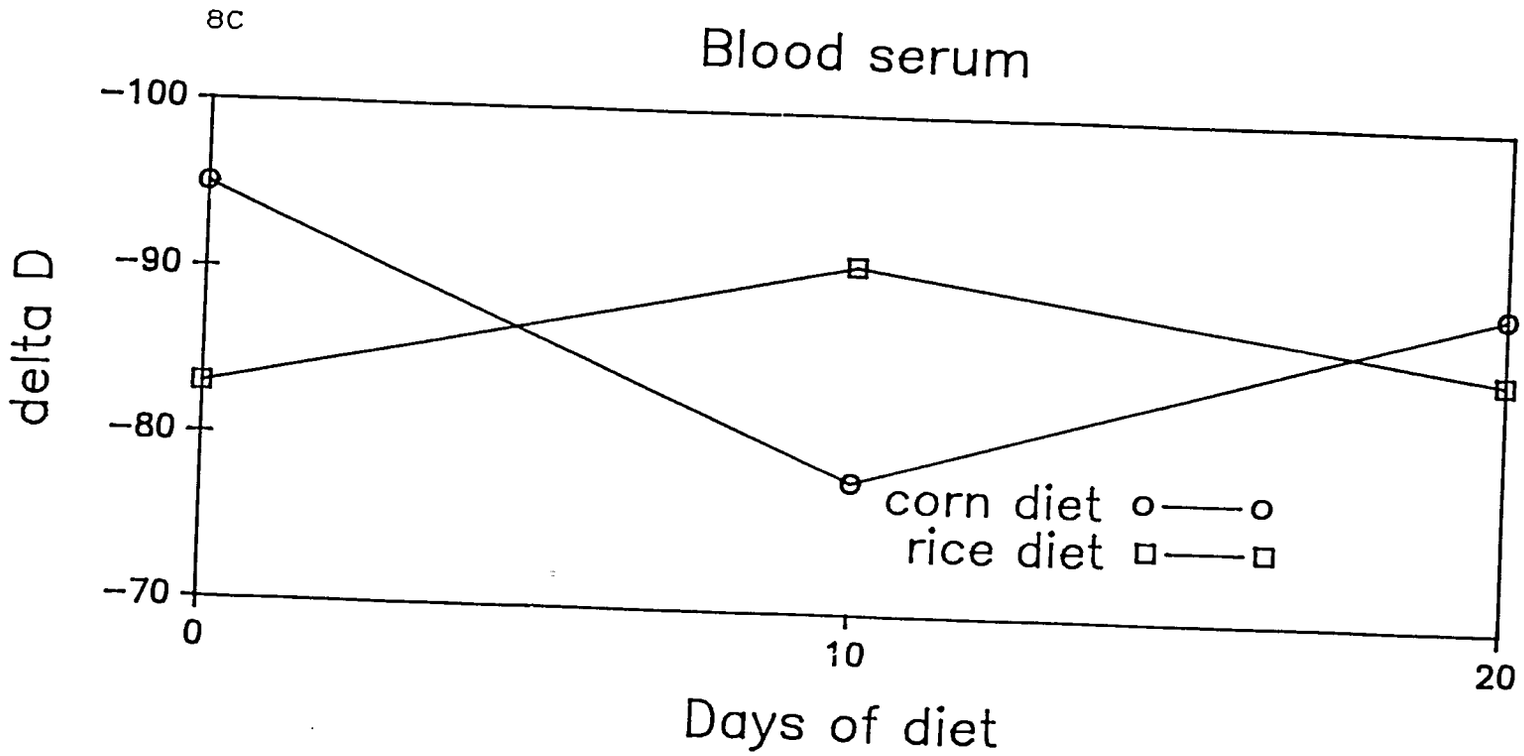
Figure 8. delta D values of selected body tissues collected from adults consuming the indicated diets for ten or twenty days.



8B


Saliva
delta D
−105
−90
−75
−60
Corn diet
delta D
−105
−90
−75
−60
Rice diet
0
4
8
12
16
20
Days of diet

8C


Blood serum
delta D
−100
−90
−80
−70
0
10
20
Days of diet
corn diet
rice diet