shaare zedek medical center המרכז הרפואי שערי צדק

U.S.-Israel CDR Program,
Room 720, SA-18,
Agency for International Development,
Washington D.C. 20523.

C7-079

May 28, 1989.

Dear Sir,

Grant number DPE-5544-G-SS-7067-00

I have pleasure in enclosing the second semiannual report. When perusing the raw data attached please note:

1. There are five subjects indicated by their initials :M, R, S, Sa, Y.
2. Following each intial there appears a number a number indicating the day of the trial.
3. The diet appropriate to the day of the trial can be ascertained from the methods section of the report.

Yours sincerely,

S.Freier.
Principal Investigator.

Rec'd in SCI: JUN 6 1989

Jerusalem 91-031 ירושלים 91-031
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PN-AGM-543
29428

U.S.-ISRAEL COOPERATIVE DEVELOPMENT RESEARCH PROOGRAM.

Second Progress Report May 1989.

Title of Research Project:

Evaluation of Triglycerides as a rapidly assimilable Energy Source for malnourished lactating Women and Children.

Grant Number: PDE-5544-G-7076-00

Principal Investigator:

Prof. S. Freier,
Chairman,
Department of Pediatrics,
Shaare Zedek Medical Center,
Jerusalem.

Cooperating Investigator:

Dr. Nieva Librojo,
Institute of Chemistry,
University of the Philippines at Los Banos,
College, Laguna,
Philippines, 4031.

Signature of Principal Investigator:

OVERALL OBJECTIVES.

To investigate, in-vivo, the rate and extent of metabolism of medium chain triglycerides (MCT), relative to that of long chain triglycerides (LCT) in properly nourished and in malnourished humans.

To evaluate the efficacy of coconut oil as a locally available source of MCT for treatment of malnutrition in the tropics.

OBJECTIVES OF SECOND SIX MONTHS.

The methodology employed is based on the differences in the ratio of the stable isotope ^{13}C to carbon ^{12}C in various foodstuffs. This ratio is referred to as delta C (δC). From the point of view of their delta C, the plant kingdom is divided into two groups: corn and pineapple, also referred to as C4 foods, having a comparatively high content of ^{13}C and all other plants having a relatively lower ^{13}C content. These plants are also referred to as the C3 plants, a term referring to the synthesis of their basic carbohydrate unit. Our theory, based on these facts was that if the body was stabilized on C4 foods, that is corn and pineapple, one could then add the C3 food to be tested and measure the effect of this food on the δC of the exhaled air. In our instance the food to be tested was MCT.

Three sets of experiments are described here:

1. To establish if the use of corn oil (which is C4) would be sufficient to produce measurable changes in the breath δC in an individual eating an otherwise normal diet. (Results see below).
2. After it had been established that the diet tested in 1. above did not produce the desired effect, one individual took a C4 diet for a period of 2 weeks. During the first week, all energy sources were of C4 derivation, while during the second week, protein and

carbohydrate were from C4 foods, while the oil imbibed was MCT (C3).

3. Using the data obtained in the second experiment, we planned a third set of tests in which three individuals would receive the C4 corn and pineapple diet on the first day, and adding to this basic diet MCT on the second day and LCT (soy oil) on the third day. In addition, two lactating mothers would take the same diet as the other three individuals, supply samples of breath and, in addition, remove specimens of foremilk at every breast feed.

INDIVIDUALS AND METHODS.

1. The first experiment was performed by one individual only. A normal diet was taken for the whole period of the experiment which lasted from August 28, 1988 thru September 9, 1988. During the first three days of the experiment 25 ml of soy oil were taken each morning. During the next 5 days of the experiment 25 ml of corn oil (C4) were taken each morning, while during the last three days 25ml of coconut oil (MCT) were taken each morning. The individual took samples of breath and stool for a period of two weeks at the times indicated on the results.

2. The second experiment was again performed by one individual only. The experiment started on January 2, 1989. For the first six days the individual ate only C4 foods, including cornflour fried in corn oil, corn, cornflakes, corncake and pineapple. Samples of stool and breath were taken as indicated in the results.

3. Three healthy adults (two male and one female) and two lactating mothers participated in the third experiment. It lasted three days.

The first day was required for establishing a base line on a diet consisting entirely on corn products, chickens raised on corn products and pineapple. The basic C4 diet was maintained on days 2 and 3, but on day 2 MCT (C3) was introduced and on day 3 LCT.

Diet day 1: Morning: Corncake containing 50 ml of corn oil.
Noon: Corn on the cob, popcorn, cornflakes.
Evening: as for noon, with corn raised chicken optional.

Diet day 2: The corncake in the morning was prepared with 50 ml MCT. Otherwise as on day 1.

Diet day 3. The corncake in the morning was prepared using 50 ml LCT (soy-oil).

All samples of breath and stool were sealed, incinerated locally, and forwarded to commercial laboratories in the United States. Samples of milk are being processed at the time of writing in our biochemistry laboratories. They are being fractionated into protein, fat and carbohydrate moieties and will be forwarded to the United States for measurement of ΔC thereafter.

RESULTS

Experiment 1. It will be noticed that the ΔC in the breath remained steady during the baseline period when the individual ate a normal C3 diet, including the C3, LCT oil derived from soy-beans. However, the introduction of corn-oil on day 5 (FS 5A) for a period of 3 days, up to and including specimen FS 8A, did not produce a change in the ΔC, and

therefore, no change was noticed in the last 4 days during which MCT, a C3 oil was introduced.

Experiment 2. Specimens of breath 1-A-a up to and including 6-A-b were obtained on a diet in which all sources of energy were derived from C4 foods. δC rose (being a negative value) from -24.3 to -18.6 (1-A-C) by four hours and reached -17 (1-A-d) 12.5 hours after starting the diet. No significant change occured during the night suggesting that the body was burning mainly fuels obtained during the previous 24 hours. By the evening of the second day (2-A-b) a further rise to -16.3 had occurred. The next 3 days showed that at night a slight regression occurred to metabolism of body stores of C3 manifested by lower (i.e. more negative) values of δC. The introduction of MCT, in the form of coconut oil, (specimens 7-A-a thru 10-A-d) did not appreciably alter the pattern previously established, where the early morning specimens indicated that the body was metabolizing a mixture of C3 and C4 fuels while in the evening metabolism of the C4 corn diet predominated in spite of the addition of coconut oil a C3 source containing about 60% of MCT.

The change of the δC in the stool was noticeable within 5 days and did not change any more for the duration of the experiment.

The conclusions of this experiment were that a few hours of a C4 diet were sufficient for obtaining a maximal effect in the δC in the breath. This effect became less at night when the body apparently also burned energy from "stock". The third experiment was planned on the basis of these data.

Experiment 3. In this experiment day 1 was devoted to establishing a base line on a diet where protein, carbohydrate and fat were derived from C4 foods. In subject S a rise (a drop in the minus value) of δC occured within 15 minutes and continued throughout the day reaching a high at 10

pm. Subjects Sa and Y showed a similar ternd. Subject M reached the highest value at the 6th hour and showed a rebound at 10 pm. The rise in subject R was less marked than in the other subjects.

The morning, fasting, value on day 2 (all specimens with the numeral 2 after the initial) was lower than on day 1 in all subjects. This was particularly marked in subjects S and Y. It will be recollected that on this day the fat source was 50 gm of MCT-oil taken at breakfast. The rise in BC on the second day was less marked. In all five subjects the effect of MCT was noticeable for 6 hours, while the 10 pm value (i.e. 12-14 hours after ingesting the MCT) the effect was no longer noticeable. The effect of LCT (all specimens with the numeral 3 after the initial) was variable. In subject S, M and Sa the effect of LCT was still noticeable at 6 hours, while in subject Y the effect of the C4 diet prevented the manifestation of the C3 oil in the breath.

CONCLUSIONS

The results of the experiments show that useful data on the metabolism of fats can be obtained by the methodology employed.

The design of the first experiment was faulty for two reasons:

1. The amount of oil ingested in the morning (25 ml) was insufficient and

2. the sampling time of the specimens was too infrequent.

The lesson learnt from the second experiment was that the desired effect in breath is obtained already on the first day of the C4 diet and in the feces on the fifth day. A single day is, therefore, sufficient for establishing a baseline for changes in in the breath, but more frequent samples are required for studying teh kinetics of the changes in the breath.

Experiment 3 showed that the C4 diet enters the metabolic pool within 60 minutes of ingestion and seems to play an ever increasing role during the course of the first day. Even after the fast of the first night its effect was still noticeable, as the δC in all individuals was higher on the second morning.

The main purpose of this research is to establish data on the metabolism of MCT. In all but one subject the effect of MCT was noticeable by 15 minutes as δC at this short interval was already higher in 4 out of 5 subjects. This effect of MCT was still noticeable for at least 6 hours, indicating that MCT enters the metabolic pool rapidly and stays there for a period of several hours. Similarly, the effect of LCT was noticeable at 15 minutes in 3 subjects and persisted throughout the day. Unfortunately, the experiment terminated after 6 hours so that the effect after 12 hours could not be observed.

We could, therefore, not detect a noticeable difference between the metabolism of LCT and MCT by the methodology employed.

FUTURE PLANS

In order to obtain reliable information we plan to introduce further refinements in the experiments which will now be continued in the Philippines.

1. In order to introduce complete standardisation, the amount of protein and carbohydrate ingested should be uniform for all individuals on all days.

2. Breakfast on all days should include the C4 corn oil. Two hours later the oil to be tested should be introduced.

3. It is probably unnecessary to take 4 samples during the first hour; two samples should be sufficient.

EXPERIMENT 3.

KRUEGER ENTERPRISES, INC.
GEOCHRON LABORATORIES DIVISION
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STABLE ISOTOPE RATIO ANALYSES

REPORT OF ANALYTICAL WORK

Submitted by: S. Freier
Chairman, Dept. of Pediatrics
Institute of Gastroenterology
Shaare Zedek Medical Center
Jerusalem 91-000
ISRAEL

Date Received: 04/10/89
Date Reported: 04/28/89
Your Reference: Freier letter Apr. 5

Day 1. PATIENT CH2 BOLUS GLUCOSE - Cu.
FAT Cu

Our Lab Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54647	S1 0'	Breath CO_2	-22.4
CR-54648	S1 15'	"	-21.4
CR-54649	S1 30'	"	-21.0
CR-54650	S1 45'	"	-20.8
CR-54651	S1 1 hr	"	-20.1
CR-54652	S1 2 hr	"	-17.9
CR-54653	S1 3 hr	"	-17.8
CR-54654	S1 4 hr	"	-17.3
CR-54655	S1 5 hr	"	-17.6
CR-54656	S1 6 hr	"	-18.1
CR-54657	S1 22 00	"	-14.5

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$$\delta^{13}C_{sample}‰ = \left[\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right] \times 1000$$

Where:

$^{13}C/^{12}C$ standard is PDB

And:

$^{13}C/^{12}C$ standard = 0.011237

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STABLE ISOTOPE RATIO ANALYSES — REPORT OF ANALYTICAL WORK

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Date Received: 04/10/89
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PROTEIN + CARBOHYDRATE C4.
FAT C3 - PURE MCT

DAY 2

Our Lab. Number	Your Sample Number	Description	$\delta^{13}C^*$
CR-54658	S2 0'	Breath CO_2	-17.7
CR-54659	S2 30'	"	-18.1
CR-54660	S2 45'	"	-17.9
CR-54661	S2 1 hr	"	-18.4
CR-54662	S2 2 hr	"	-18.7
CR-54663	S2 3 hr	"	-19.5
CR-54664	S2 4 hr	"	-19.9
CR-54665	S2 5 hr	"	-20.4
CR-54666	S2 6 hr	"	-20.7
CR-54667	S2 22 00	"	-16.9

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Where:
$^{13}C/^{12}C$ standard is PDB

And:
$^{13}C/^{12}C$ standard = 0.011217

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STABLE ISOTOPE RATIO ANALYSES

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PROTEIN
CARBOHYDRATE } C4
FAT C3 -- Soy oil

DAY 3.

Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54668	S3 0'	Breath CO_2	-17.1
CR-54669	S3 15'	"	-17.6
CR-54670	S3 30'	"	-17.8
CR-54671	S3 45'	"	-18.2
CR-54672	S3 1 hr	"	-17.9
CR-54673	S3 2 hr	"	-18.2
CR-54674	S3 3 hr	"	-18.9
CR-54675	S3 4 hr	"	-19.5
CR-54676	S3 5 hr	"	-19.9
CR-54677	S3 6 hr	"	-20.0

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Where:
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And:
$^{13}C/^{12}C$ standard = 0.0112:17

SPECIALISTS IN GEOCHRONOLOGY & ISOTOPE GEOLOGY

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ISRAEL

Date Received: 04/10/89
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Regarding for S1

Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54584	M1 0'	Breath CO_2	-24.5
CR-54585	M1 15'	"	-24.5
CR-54586	M1 30'	"	-24.0
CR-54587	M1 45'	"	-22.8
CR-54588	M1 60'	"	-22.1
CR-54589	M1 2h	"	-20.4
CR-54590	M1 3h	"	-19.0
CR-54591	M1 4h	"	-18.2
CR-54592	M1 5h	"	-17.3
CR-54593	M1 6h	"	-16.0
CR-54594	M1 22h	"	-19.1

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And:
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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54595	M2 0'	Breath CO_2	-21.8
CR-54596	M2 15'	"	-22.0
CR-54597	M2 30'	"	-21.8
CR-54598	M2 45'	"	-21.7
CR-54599	M2 1 hr	"	-21.7
CR-54600	M2 2 hr	"	-20.1
CR-54601	M2 3 hr	"	-20.1
CR-54602	M2 4 hr	"	-20.5
CR-54603	M2 5 hr	"	-21.1
CR-54604	M2 6 hr	"	-21.8
CR-54605	M2 2200	"	-18.7

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Requested on for $\delta^{13}C$

Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54606	M3 0'	Breath CO_2	-22.6
CR-54607	M3 15'	"	-22.4
CR-54608	M3 30'	"	-22.3
CR-54609	M3 45'	"	-21.8
CR-54610	M3 60'	"	-21.5
CR-54611	M3 2 hr	"	-21.2
CR-54612	M3 3 hr	"	-21.0
CR-54613	M3 4 hr	"	-19.8
CR-54614	M3 5 hr	"	-19.4
CR-54615	M3 6 hr	"	-19.7

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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54616	R1 0'	Breath CO_2	-24.2
CR-54617	R1 15'	"	-23.6
CR-54618	R1 30'	"	-23.6
CR-54619	R1 45'	"	-23.1
CR-54620	R1 1 hr 15'	"	-22.0
CR-54621	R1 2 hr	"	-20.8
CR-54622	R1 3 hr	"	-20.9
CR-54623	R1 4 hr	"	-21.3
CR-54624	R1 5 hr	"	-21.7
CR-54625	R1 6 hr	"	-21.6
CR-54626	R1 22 00	"	-20.2

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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54627	R2 0'	Breath CO_2	-21.5
CR-54628	R2 15'	"	-21.1
CR-54629	R2 30'	"	-21.1
CR-54630	R2 45'	"	-21.7
CR-54631	R2 1 hr	"	-21.8
CR-54632	R2 2 hr	"	-21.6
CR-54633	R2 3 hr	"	-21.1
CR-54634	R2 4 hr	"	-20.8
CR-54635	R2 5 hr	"	-21.6
CR-54636	R2 6 hr	"	-22.7
CR-54637	R2 22 00	"	-21.5

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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54638	R3 0'	Breath CO_2	-21.6
CR-54639	R3 30'	"	-21.5
CR-54640	R3 45'	"	-21.2
CR-54641	R3 1 hr	"	-21.0
CR-54642	R3 2 hr	"	-20.5
CR-54643	R3 3 hr	"	-20.4
CR-54644	R3 4 hr	"	-20.7
CR-54645	R3 5 hr	"	-21.8
CR-54646	R3 6 hr	"	-21.8

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Date Received: 04/10/89
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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54678	Sa1 0'	Breath CO_2	-22.8
CR-54679	Sa1 15'	"	-21.5
CR-54680	Sa1 30'	"	-21.0
CR-54681	Sa1 45'	"	-20.6
CR-54682	Sa1 1 hr	"	-19.7
CR-54683	Sa1 2 hr	"	-17.6
CR-54684	Sa1 3 hr	"	-20.1
CR-54685	Sa1 4 hr	"	-15.8
CR-54686	Sa1 5 hr	"	-16.6
CR-54687	Sa1 6 hr	"	-17.1
CR-54688	Sa1 22 00	"	-14.9

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- Breakfast only for Sa2

Our Lab. Number	Your Sample Number	Description	$\delta^{13}C^*$
CR-54689	Sa2 0'	Breath CO_2	-18.7
CR-54690	Sa2 15'	"	-19.0
CR-54691	Sa2 30'	"	-19.2
CR-54692	Sa2 45'	"	-19.1
CR-54693	Sa2 1 hr	"	-19.1
CR-54694	Sa2 2 hr	"	-18.4
CR-54695	Sa2 3 hr	"	-19.1
CR-54696	Sa2 4 hr	"	-19.6
CR-54697	Sa2 6 hr	"	-16.9
CR-54698	Sa2 7 hr (?)	"	-16.1
CR-54699	Sa2 22 00	"	-18.1

*Unless otherwise noted, analyses are reported in ‰ notation and are computed as follows:

$$\delta^{13}C_{sample}‰ = \left[\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right] \times 1000$$

Where: $^{13}C/^{12}C$ standard is PDB

And: $^{13}C/^{12}C$ standard = 0.011237

KRUEGER ENTERPRISES, INC.
GEOCHRON LABORATORIES DIVISION
24 BLACKSTONE STREET • CAMBRIDGE, MASSACHUSETTS 02139 • (617) 876-3691

STABLE ISOTOPE RATIO ANALYSES

REPORT OF ANALYTICAL WORK

Submitted by: S. Freier
Chairman, Dept. of Pediatrics
Institute of Gastroenterology
Shaare Zedek Medical Center
Jerusalem 91-000
ISRAEL

Date Received: 04/10/89
Date Reported: 05/04/89
Your Reference: Freier letter Apr. 5

Legend on file 53

Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54700	Sa3 0'	Breath CO_2	-21.5
CR-54701	Sa3 15'	"	-22.0
CR-54702	Sa3 30'	"	-21.8
CR-54703	Sa3 45'	"	-21.4
CR-54704	Sa3 1 hr	"	-21.7
CR-54705	Sa3 2 hr	"	-20.2
CR-54706	Sa3 3 hr	"	-20.0
CR-54707	Sa3 4 hr	"	-20.4
CR-54708	Sa3 5 hr	"	-20.4
CR-54709	Sa3 6 hr	"	-18.5

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Where:
$^{13}C/^{12}C$ standard is PDB

And:
$^{13}C/^{12}C$ standard = 0.011237

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STABLE ISOTOPE RATIO ANALYSES — REPORT OF ANALYTICAL WORK

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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54710	Y1 0'	Breath CO_2	-23.7
CR-54711	Y1 15'	"	-21.1
CR-54712	Y1 30'	"	-20.1
CR-54713	Y1 45'	"	-19.2
CR-54714	Y1 1 hr	"	-18.6
CR-54715	Y1 2 hr	"	-17.2
CR-54716	Y1 3 hr	"	-16.8
CR-54717	Y1 4 hr	"	-16.3
CR-54718	Y1 5 hr	"	-16.3
CR-54719	Y1 6 hr	"	-16.4
CR-54720	Y1 22 00	"	-15.4

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Where: $^{13}C/^{12}C$ standard is PDB

And: $^{13}C/^{12}C$ standard = 0.011237

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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54721	Y2 0'	Breath CO_2	-17.2
CR-54722	Y2 15'	"	-17.4
CR-54723	Y2 30'	"	-17.2
CR-54724	Y2 45'	"	-17.5
CR-54725	Y2 1 hr	"	-17.3
CR-54726	Y2 2 hr	"	-16.3
CR-54727	Y2 3 hr	"	-16.1
CR-54728	Y2 4 hr	"	-16.2
CR-54729	Y2 5 hr	"	-17.0
CR-54730	Y2 6 hr	"	-17.8
CR-54731	Y2 22 00	"	-14.6

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Where:

$^{13}C/^{12}C$ standard is PDB

And:

$^{13}C/^{12}C$ standard = 0.011237

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Our Lab. Number	Your Sample Number	Description	$\delta^{13}C$*
CR-54732	Y3 0'	Breath CO_2	-16.2
CR-54733	Y3 15'	"	-16.4
CR-54734	Y3 30'	"	-16.2
CR-54735	Y3 45'	"	-16.0
CR-54736	Y3 1 hr	"	-15.4
CR-54737	Y3 2 hr	"	-14.3
CR-54738	Y3 3 hr	"	-14.5
CR-54739	Y3 4 hr	"	-14.2
CR-54740	Y3 5 hr	"	-14.0
CR-54741	Y3 6 hr	"	-14.3
CR-54742	Y3 22 00	"	-17.5

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$$\delta^{13}C_{sample}‰ = \left[\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right] \times 1000$$

Where:

$^{13}C/^{12}C$ standard is PDB

And:

$^{13}C/^{12}C$ standard = 0.011237

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