1972

Study of the metabolism of trimethylpropylsilane

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A STUDY OF THE METABOLISM OF TRIMETHYLPROPYLSILANE

By

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B. S. Jacksonville University, 1966

Presented in partial fulfillment of the requirements
for the degree of

Master of Science

UNIVERSITY OF MONTANA

1972

Approved by:

Chairman, Board of Examiners

Dean, Graduate School

Date June 6, 1972

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ACKNOWLEDGEMENTS

I wish to express my gratitude to my adviser, Dr. R. J. Fessenden, for his guidance through this research and the preparation of this thesis.

I also wish to thank Dr. W. L. Koostra for the use of his scintillation counter and Mike Gransbery for his aid in preparing the compounds.

And, of course, I am especially grateful to my wife, Fran, for her patience and continued encouragement.

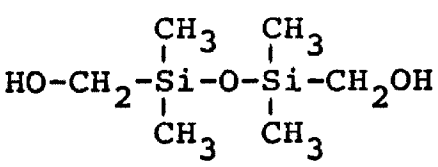
CHAPTER I

INTRODUCTION

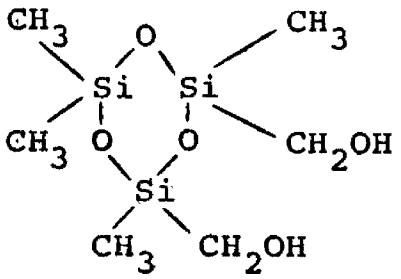
To date, little has been reported on the metabolic end products of organosilicon compounds. It has been shown[2] that certain silicon-containing carbamates undergo dealkylation in the rat. Evidence has been found[1] which indicates that phenylsilanes undergo hydroxylation before they are eliminated.

Literature reference to the metabolism of tetraalkylsilanes is limited to the work of Paul and Pover,[8] who have provided some evidence that trimethylsilylhexadecane is absorbed from the gastrointestinal tract of rats.

Previous experiments in this laboratory[4] suggest that trimethylpropylsilane undergoes oxidation and dealkylation to produce a variety of products, including I and II.



I



II

Since silicon has no radioactive isotope which lends itself to useful labeling techniques, the practice for

tracer studies of the metabolism of alkylsilanes has been to use a C^{14} label in an alpha position. However, if dealkylation is occurring, as suggested by structures I and II, then counting data cannot be considered as indicative of the tetraalkylsilanes, since the bond between the silicon and the tracer element does not remain intact.

However, it had not been determined if this bond were being broken *in vivo* or if, indeed, the conditions of extraction of the urine were causing dealkylation.

$$R_3SiCH_3 \xrightarrow{\text{rat}} R_3Si\text{-}O\text{-}$$

$$R_3SiCH_3 \xrightarrow{\text{rat}} R_3SiCOOH \xrightarrow{\text{extraction}} R_3Si\text{-}O\text{-}$$

Research goals

The purpose of this research was to study the metabolism of trimethylpropylsilane in the rat to determine if the breaking of the silicon-methyl bond occurs in the metabolic pathway of the rat or if it might be an artifact of the laboratory work-up.

In the previous study of this compound, C^{14}-trimethylpropylsilane was administered to rats and the urine treated to isolate the radioactive material. The urine was extracted with ethyl acetate or diethyl ether and the solvent was evaporated in order to concentrate the extract. Separation

was effected by successive applications of thin layer chromatography.

By following this procedure using a doubly labeled compound, it was hoped that the point at which dimethylation was occurring might become apparent.

Structures I and II suggest that the first step in the dealkylation might be oxidation of the methyl group. If the oxidation proceeded to the carboxyalkylsilane, then on the basis of chemical analogy,[7] in vitro decarboxylation could be expected during the work-up procedure.

$$R_3SiCH_3 \xrightarrow{rat} R_3SiCOOH \xrightarrow{H_2O} R_3Si\text{-}O\text{-} + CO_2$$

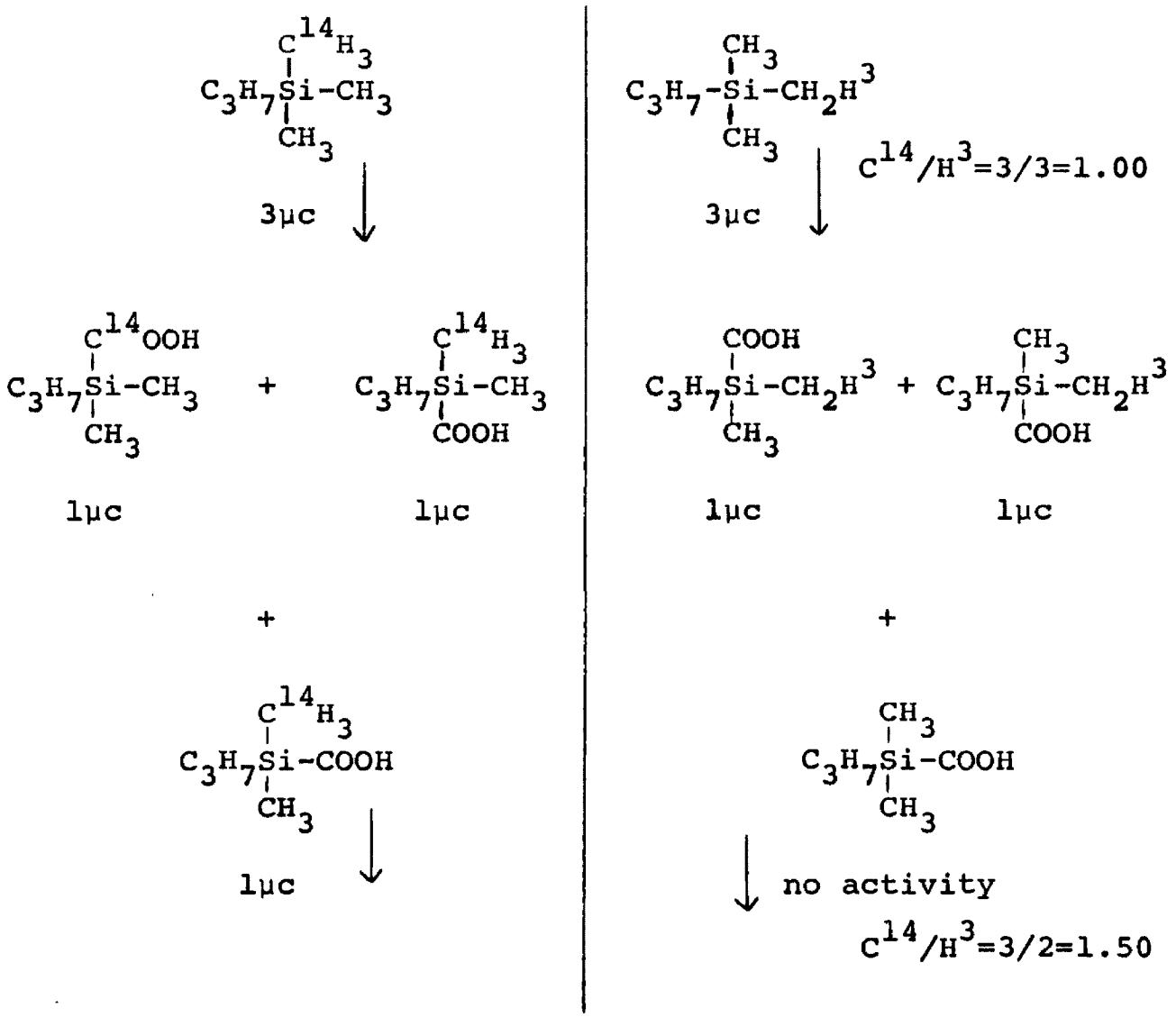
If so, then some insight into the point where the methyl group is being lost could be gained by examining the ratio of the two isotopes C^{14} and H^3, both on methyl groups (see Scheme I).

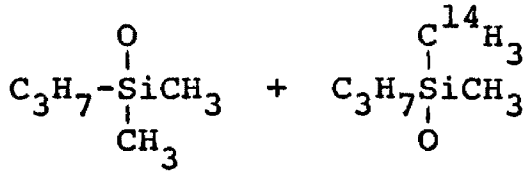
Assume the two isotopes are present in the same activity level at the start. When oxidation occurs, the probability is 1/3 that the methyl group oxidized will be the one with the H^3 label. This would produce a change in the ratio of the two isotopes C^{14}/H^3 from 3/3 to 3/2, or from 1.00 to 1.50. Subsequently, when the decarboxylation occurs, the chances are again 1/3 that the C^{14} label would be lost, thus the C^{14}/H^3 ratio would return to 1.00. Thus,

if this pathway was in operation, then the eliminated urine should show a higher C^{14} to H^3 ratio which should return to its original level after extraction. If the demethylation occurs <u>in vivo</u>, then the ratio would not be expected to change.

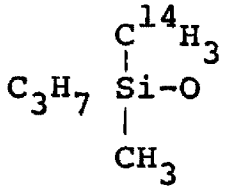
SCHEME I

OUTLINE OF TRACER STUDY OF C^{14}-H^3-TRIMETHYLPROPYLSILANE

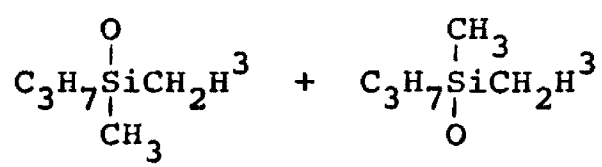




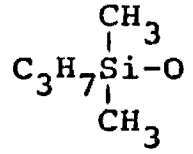
no activity 1μc



1μc



1μc 1μc



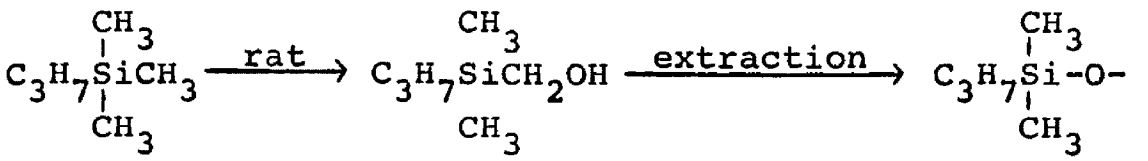
$C^{14}/H^3 = 2/2 = 1.00$

no activity

It should be noted that the presence of hydroxymethyl groups in structures I and II suggest an alternative pathway for demethylation. That is, there may be only limited oxidation to the alcohol before the methyl group is lost. This is outlined in Scheme II. Since there is one methyl hydrogen being replaced out of a possible nine positions, the H^3 activity would be expected to be reduced to 8/9 of its initial level. The C^{14}/H^3 ratio would then be 9/8 = 1.125. If this is the case, the ratio in the eliminated urine should be 1.125 and should reduce to 1.00 after extraction.

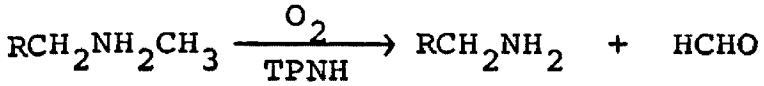
SCHEME II

ALTERNATIVE PATHWAY FOR DEMETHYLATION OF TRIMETHYLPROPYLSILANE



C^{14}/H^{3}=3/3=1.00 =9/8=1.125 =2/2=1.00

A further possibility is that of direct methyl transfer. This has been shown to be a detoxification pathway in the case of N-methyl groups.[9] If this pathway is in operation, the ratio would not be expected to change.



Synthesis outline

The use of Grignard reagents to form C-Si bonds is well known.[11] n-Propylmagnesium chloride was prepared and its reaction with dimethyldichlorosilane gave n-propyldimethylchlorosilane. Reaction of this compound with C^{14}-methyl Grignard reagent produced C^{14}-trimethylpropylsilane in high yield. This is outlined in Scheme III.

SCHEME III

SYNTHESIS OF C^{14}-TRIMETHYLPROPYLSILANE

$$C_3H_7\text{-}Cl + Mg \xrightarrow[I_2]{\text{ether}} C_3H_7MgCl \xrightarrow{(CH_3)_2SiCl_2} (CH_3)_2\overset{\overset{C_3H_7}{|}}{Si}Cl$$

$$C^{14}H_3I + Mg \xrightarrow{\text{ether}} C^{14}H_3MgI \xrightarrow{(CH_3)_2\overset{\overset{C_3H_7}{|}}{Si}Cl} (CH_3)_2\overset{\overset{C_3H_7}{|}}{Si}C^{14}H_3$$

Trimethylchlorosilane was irradiated with ultraviolet light in the presence of chlorine gas to produce (chloromethyl)dimethylchlorosilane. This was treated with n-propyl Grignard reagent to give (chloromethyl)dimethylpropylsilane. The corresponding Grignard reagent was prepared and hydrolyzed with H^3-water to produce H^3-trimethylpropylsilane. This is outlined in Scheme IV.

SCHEME IV

SYNTHESIS OF H^3-TRIMETHYLPROPYLSILANE

$$(CH_3)_3Cl + Cl_2 \xrightarrow{u.v.} (CH_3)_2\overset{\overset{CH_2Cl}{|}}{Si}Cl \xrightarrow{C_3H_7MgBr} (CH_3)_2\overset{\overset{CH_2Cl}{|}}{Si}C_3H_7$$

$$Mg + (CH_3)_2\overset{\overset{CH_2Cl}{|}}{Si}C_3H_7 \xrightarrow{\text{ether}} (CH_3)_2\overset{\overset{CH_2MgCl}{|}}{Si}C_3H_7 \xrightarrow{H_2^3O} (CH_3)_2\overset{\overset{CH_2H^3}{|}}{Si}C_3H_7$$

CHAPTER II

EXPERIMENTAL

The following symbols and procedures were used consistently throughout this chapter and are assumed unless otherwise noted. All temperatures are in °C and pressures in mm Hg. All rats used were 100-200 g male Long-Evans rats purchased from Simonsen Laboratories, Gilroy, California. Trimethylchlorosilane and dimethyldichlorosilane were purchased from Union Carbide Corp. and redistilled before using. n-Propyl chloride and methyl iodide were purchased from Aldrich Chemical Co., Inc. 1-Bromopropane was purchased from J. T. Baker Chemical Co. Water-H^3 and methyl iodide-C^{14} were purchased from New England Nuclear Corp.

Gas chromatographic samples were analyzed using an Aerograph A-700. Detection was by thermal conductivity. Infrared spectra were obtained using a Beckman IR-5a spectrophotometer and were run as thin film samples. All nuclear magnetic resonance spectra were obtained using a Varian HA-60 nmr spectrometer as 10% solutions in carbon tetrachloride. Benzene at 7.37 ppm downfield from tetramethylsilane was used as an internal standard. Radioactive samples were counted on a Packard Tri-Carb liquid scintillation counter and on a Nuclear Chicago Unilux III

liquid scintillation counter. The fluor solution was a toluene:ethanol mixture, 80:20 by volume with 4.4 g of Packard Permablend M per liter of solution.

n-Propyldimethylchlorosilane

In a 2-l 3-necked round bottom flask was placed 14.0 g (0.576 mole) of magnesium turnings. The flask was equipped with a reflux condenser, a mechanical stirrer, a calcium chloride drying tube and an addition funnel. Diethyl ether was added in sufficient quantity to cover the magnesium, and 39.4 g (0.5 mole) of n-propyl chloride mixed with an equivalent volume of ether was added dropwise through the funnel. Addition of a crystal of iodine was necessary to initiate the reaction and the flask was heated to maintain reflux during the addition. Reflux was continued for thirty minutes after the addition was complete and 96.0 g (0.742 mole) of dimethyldichlorosilane was added to the reaction mixture as quickly as possible. The reaction was refluxed until the color of the Grignard was gone. The magnesium salts were removed by suction filtration and centrifugation and the supernatant was washed with petroleum ether (30°-60°). The ether was evaporated under vacuum and the product was separated by vacuum distillation on a 40 cm Vigreaux column. Fractions were collected

between 70° and 78° (160 mm) and analyzed for purity by gas chromatography. Co-injection of all fractions showed that they were the same compound, and no impurities could be detected by the chromatographic analysis. A thin film sample was subjected to analysis by IR spectrophotometry. Major absorption bands appear at 2950 cm^{-1}, 1225 cm^{-1}, 1080 cm^{-1}, and a broad band between 793 cm^{-1} and 846 cm^{-1}. Total yield was 15.2 g (22.4%).

C^{14}-Trimethylpropylsilane

In a 250 ml 3-necked round bottom flask was placed 3.6 g (0.148 mole) of magnesium turnings and enough diethyl ether to cover the magnesium. The flask was then fitted with a reflux condenser with a calcium chloride drying tube, a mechanical stirrer and an addition funnel. To this was added 1.0 g of methyl iodide to initiate the reaction. A mixture containing 17.4 g of methyl iodide and 1.4 g of C^{14}-methyl iodide was prepared and diluted to 100 ml with ether. The C^{14}-methyl iodide was found to have a radioactivity level of 73.5 c/g. The calculation of the radioactivity is as follows:

Average cpm of a 1λ aliquot = 265,540

265,540 cpm x 1.4 dpm/cpm = 371,760 dpm

$$\frac{371{,}760 \text{ dpm}}{2.22 \times 10^{6} \text{ dpm}/\mu c} = 1.6740 \times 10^{-1} \ \mu c$$

$$1.6740 \times 10^{-1} \ \mu c/\lambda = 167.40 \ \mu c/ml$$

$$\frac{1.67.40 \ \mu c/ml}{2.28 \ g/ml} = 73.5 \ \mu c/g$$

This mixture was added dropwise through the funnel and the reaction rate was controlled by the use of an ice bath. When the addition was complete and the magnesium had lost its luster, the reflux was continued for another 2 hours. A mixture of 15.2 g (0.112 mole) of propyldimethylchlorosilane mixed with about 50 ml of ether was then added to the reaction vessel while maintaining a controlled reflux rate with the ice bath. When the addition was complete, the solution was refluxed for an additional 2 hours. Hydrochloric acid (0.05 M) was then added to dissolve the magnesium salts and hydrolyze any unreacted Grignard reagent and chlorosilane. The solution was then transferred to a separatory funnel and the aqueous layer was separated from the organic and washed with two 20 ml portions of ether. The combined layer was washed with 10 ml of water and 20 ml of a solution of sodium thiosulfate (8 g in 50 ml) to convert any iodine to iodide ion. Washing with silver nitrate solution (4.3 g in approximately 30 to 40 ml of water) precipitated the iodide. Silver iodide was removed by suction filtration and the ether layers were combined

and dried over sodium sulfate overnight. The product was separated by fractional distillation on a 40 cm Vigraux column and analyzed by gas chromatography and IR spectrophotometry. Fractions boiling between 75° and 86° exhibited only one detectable compound upon co-injection in the gas chromatograph. An IR spectrum of a thin film sample had major absorption bands at 2950 cm^{-1}, 1240 cm^{-1}, 1060 cm^{-1}, 752 cm^{-1}, 688 cm^{-1} and a broad band centered at 841 cm^{-1}. This spectrum was identical to the IR spectrum of a sample of trimethyl propyl silane, the structure of which had previously been determined.[10] The activity of the sample was calculated as follows:

Average cpm of a 2.0 λ sample = 19.396

$19{,}396 \times 1.4 = 27{,}154$ dpm

$$\frac{27{,}154 \text{ dpm}}{2.22 \times 10^{6} \text{ dpm}/\mu c} = 1.22 \times 10^{-2} \ \mu c$$

$$\frac{0.0122 \ \mu c}{2.0 \times 10^{-3} \text{ ml}} = 6.1 \ \mu c/ml$$

$$\frac{6.1 \ \mu c/ml}{0.70 \ g/ml} = 8.7 \ \mu c/g$$

(Chloromethyl)dimethylchlorosilane

In a 2-l 3-necked round bottom flask was placed 1085 g (10.0 mole) of trimethylchlorosilane. The flask was fitted with a mechanical stirrer, a reflux condenser

cooled by a salt-ice bath, and a gas inlet tube. Chlorine gas was introduced through the gas inlet tube until the chlorine concentration was sufficient to turn the solution a pale green color. The gas valve was then shut off and the gas inlet tube was removed and replaced by a glass stopper. The flask was then irradiated with a General Electric 275 watt ultraviolet sun lamp until the color faded. In an effort to minimize the possibility of chlorine fire, the light was never on at the same time as the gas was being introduced. The lamp was then removed and the gas inlet tube was fitted again. The process was repeated in this fashion several times. The irradiation time required to react all the chlorine in the flask decreased with successive repetitions until it was noticed that the addition of chlorine gas no longer colored the solution. The gas was bubbled through the solution until sufficient chlorine had reacted.

To minimize the amount of multiple chlorination, the uptake of chlorine was monitored periodically, by weighing the flask. When the mass had increased by 142 g (4 g-atoms of chlorine), the reaction was stopped and the mixture was distilled on a column 60 cm long filled with glass helices 0.5 cm in diameter. Unreacted starting material was recovered at 55°-60° and returned to the reaction vessel for continued chlorination. Fractions boiling from

110°-118° were collected and analyzed. Only one compound could be detected by gas chromatography, and this was subjected to analysis by nmr. The nmr spectrum had only two peaks, one at 2.25 ppm downfield from tetramethylsilane and one at 0.25 ppm downfield. The integration ratio was 1:3. Benzene at 7.37 ppm downfield was used as the internal standard.

By repeating the process until no more starting material remained, 729 g of product were isolated (50.9%).

(Chloromethyl)dimethylpropylsilane

In a 1-l 3-necked round bottom flask equipped with a reflux condenser, a mechanical stirrer, a calcium chloride drying tube and a 500 ml addition funnel was placed 106.5 g (0.744 mole) of (chloromethyl)dimethylchlorosilane. To this was added 110 g (0.744 mole) of n-propylmagnesium bromide diluted with an equal volume of ether. The reaction mixture was stirred while the halide was added dropwise. A white precipitate of magnesium chlorobromide formed immediately and the solution was refluxed gently. When addition was complete, the stirring was continued until the reflux had subsided (about 25 min). The magnesium salts were removed by suction filtration leaving a clear, colorless liquid. The ether was removed under

vacuum and the remaining solution was distilled on a platinum spinning band column 45 cm long. A series of fractions boiling from 140°-155° was collected and analyzed. There was only one detectable compound by gas chromatography and this compound was analyzed by nmr. The nmr spectrum had a singlet at 2.58 ppm downfield from tetramethylsilane, a complicated splitting pattern centered at 0.96 ppm downfield and a singlet at 0.00 ppm. The integration ratio was 2:7:6. Total yield was 81.0 g (72.3%).

Trimethylpropylsilane-H^3

In a 100 ml 3-necked round bottom flask was placed 0.825 g (0.034 mole) of magnesium turnings. The flask was equipped with a reflux condenser, a magnetic stirrer, a calcium chloride drying tube and an addition funnel. Enough diethyl ether was added to cover the magnesium and 5.00 g (0.033 mole) of (chloromethyl)dimethylpropylsilane in diethyl ether was added dropwise. The solvent refluxed slowly. When the addition was complete, stirring was continued until the reflux stopped. Presence of an organomagnesium compound was confirmed by Gilman's test number 1.[6] The system was then placed in an ice bath and 200 λ of water-H^3 (1.00 millicurie/g) was added dropwise through the addition funnel. When it was evident

the reaction had stopped, sufficient water to complete the hydrolysis was added. Dilute hydrochloric acid (0.05 M) was added in sufficient quantity to dissolve the magnesium salts. The contents of the flask were rinsed into a 100 ml separatory funnel and the aqueous phase was separated from the organic and extracted with three 10 ml portions of ether. The extracts were combined in a distilling flask fitted with a 160 mm Vigreaux column and the ether was distilled off. The distillation was stopped at this point to add 5 ml of xylene to act as a chaser to aid in distilling the product. Fractions collected from 75°-82° were combined and analyzed. No impurities were detectable by gas chromatography. Nmr analysis was conducted and the spectrum had a complicated splitting pattern centered at 0.99 ppm downfield from tetramethylsilane and a singlet at 0.00 ppm. The integration ratio was 7:9. A total of 3.08 g were isolated (78.0%). The IR spectrum was characterized by strong, sharp absorption bands at 2950 cm^{-1}, 1240 cm^{-1}, and 1060 cm^{-1}; and a band extending from 825 cm^{-1} to 860 cm^{-1}. This spectrum was identical to the IR spectrum of a sample of trimethyl propyl silane, the structure of which had previously been determined.[10] The radioactivity of the compound was determined as follows:

H^3 standard: 50 gave 34,639 cpm

(H^3 - toluene, 106,000 dpm)

$$\text{Efficiency} = \frac{34,639}{106,000} = 0.3375 = 33.75\%$$

5.0 λ of H^3 - trimethylpropylsilane gave 133,000 cpm

$$\frac{133,000}{0.3375} = 395,000 \text{ dpm}$$

$$\frac{0.395 \times 10^6 \text{ dpm}}{2.22 \times 10^6 \text{ dpm/μc}} = 0.178 \text{ μc}$$

$$\frac{0.178 \text{ μc}}{0.005 \text{ ml}} = 35.6 \text{ μc/ml}$$

$$\frac{35.6 \text{ μc/ml}}{0.70 \text{ g/ml}} = 50.8 \text{ μc/g}$$

Dosing and Collection

A total of 18 rats were dosed orally with a mixture of the two isotopically labeled compounds. The rats used were all male Long-Evans rats and were maintained on a diet of Purina laboratory chow and water. They were dosed with from 0.25 - 0.40 ml of the mixture, which was placed directly into the stomach of the test animal using a stomach tube.

The animals were housed separately in metabolism cages purchased from Hoeltge, Inc., constructed in such a way that urine and fecal material could be collected over a 72 hr period. It was noticed that no detectable amounts of radiation were still in the urine after this time, and that reliable levels were detectable up to

36 hours after dosing.

Counting and Calculations

Before dosing on each occasion, an aliquot of the dosing mixture was counted to determine the initial ratio of C^{14} to H^3 events in the mixture. An aliquot of each of the urine samples was counted and the ratio of the two isotopes was compared to the ratio of the dosing mixture. The scintillation counter was set up for the simultaneous counting of two isotopes according to the method described by Bush.[1] The machine was standardized using aliquots of known standards of the two isotopes and the discriminator windows were adjusted in such a way that the higher energy channel counted C^{14} events only. This is labeled Channel B in Fig. 1. The efficiency of the instrument under these conditions was then calculated. The fraction of total C^{14} events appearing on Channel A was noted and the calculation carried out as follows:

$$\frac{\text{cpm of } H^3 \text{ recorded on Channel A}}{\text{dpm of standard } H^3 \text{ aliquot}} = H^3 \text{ Efficiency}$$

$$\frac{\text{cpm of } C^{14} \text{ recorded on Channel B}}{\text{dpm of standard } C^{14} \text{ aliquot}} = C^{14} \text{ Efficiency}$$

$$\frac{\text{cpm } C^{14} \text{ on Channel A}}{\text{dpm } C^{14} \text{ on Channel B}} = C^{14} \text{ overlap}$$

Then, to determine the activity of a mixed sample:

$$\frac{\text{cpm recorded on Channel B}}{C^{14} \text{ efficiency}} = \text{dpm } C^{14}$$

(cpm recorded on Channel B) x (C^{14} overlap)
= cpm C^{14} recorded on Channel A

(Channel A cpm) - (C^{14} cpm on Channel A)
= cpm H^3 recorded on Channel A

$$\frac{\text{cpm } H^3 \text{ recorded on Channel A}}{H^3 \text{ efficiency}} = \text{dpm } H^3$$

Sample calculation

Instrument Print-outs

	Channel A	Channel B
vial with C^{14} only (22,900 dpm)	3,302	13,679
vial with H^3 only (106,000 dpm)	34,639	0
vial with mixed sample	2,798	3,946

C^{14} efficiency

$$\frac{13,679}{22,900} = 59.6\%$$

H^3 efficiency

$$\frac{34,639}{106,000} = 33.75\%$$

C^{14} overlap

$$\frac{3,302}{13,679} = 24.05\%$$

dpm in unknown sample vial

dpm C^{14}

$$\frac{3,946}{0.596} = 6,620 \text{ dpm } C^{14}$$

C^{14} overlap

$$(3,946) \times (0.2405) = 950$$

H^3 dpm

$$(2,798) - (950) = 1,848$$

$$\frac{1,848}{0.3375} = 5480 \text{ dpm}$$

$$C^{14}/H^3 = \frac{6620}{5480} = 1.21$$

Calculations

Each radioactive sample was counted for 10 minutes and the disintegration rate for each of the isotopes was determined. This process was repeated 9 or 10 times and the results were tested for their statistical acceptability. Since each group of data is a random sample from a large population, the formula

$$\sigma^2 = \frac{\sum_{i=1}^{n} (x_i-\mu)^2}{(n-1)}$$

would apply, where σ is the standard deviation, n is the number of members in each sample, and μ is the arithmetic mean of the sample, calculated by:

$$\mu = \frac{\sum_{i=1}^{n} (x_i)}{n}$$

The statistical calculations were carried out by electronic data processing, using an International Business Machines computer, model 1620. A FORTRAN computer program was written for this purpose and it is shown in Table 1.

Table 1

FORTRAN Computer Program
for Calculation of Standard Deviation

```
    DIMENSION X(100)
10  READ 2,N,MM
    DO 20 J=1, N
20  READ 3, X(J)
    SX = 0.0
    DO 30 J=1,N
30  SX = SX + X(J)
    ANUM = N
    AMEAN = SX/ANUM
    SY = 0.0
    DO 40 J=1,N
40  SY = ABS(X(J) - AMEAN)**2 + SY
    AVAR = SY/(ANUM - 1.)
    ASIG = SQRT(AVAR)
    PUNCH 4, ASIG
    B= AMEAN - ASIG
    C= AMEAN + ASIG
    D= AMEAN - (2.*ASIG)
    E= AMEAN + (2.*ASIG)
    PUNCH 4,MM
    DO 50 J = 1,N
    Z = AMEAN - X(J)
50  PUNCH 4, Z
    PUNCH 5, AMEAN,B,C,D,E
    GO TO 10
 2  FORMAT (2I10)
 3  FORMAT (F10.0)
 4  FORMAT (E20.8)
 5  FORMAT(6HAMEAN=,2X,E16.8/2HB=,6X,E16.8/2HC=,6X,E16.8/
                    2HD=,6X,E16.8
   1/2HE,6X,E16.8)
60  CALL EXIT
    END
```

The program was designed to carry out the calculation of standard deviation and print out μ (AMEAN), σ(ASIG), $\mu-\sigma$(B), $\mu+\sigma$(C), $\mu-2\sigma$(D) and $\mu+2\sigma$(E). The range from D to E is then a representation of $\mu\pm2\sigma$.

This then gives chances of better than 95% that the mean of the entire population falls between D and E.

The number MM is simply an identification device so that any given group of data can be located on the print out. The value of (μ-x) for each x was printed (Z). This was to determine if one entry differed considerably from the others and was having an adverse effect on the calculation. This could then be examined as a possible source of error.

In order to test the program to be certain the calculation was being carried out properly, a set of test data was constructed and the statistical parameters were calculated by conventional methods and compared to the computer calculation. Table 2 contains this test data and the calculation follows.

Table 2

Data to Test Standard Deviation Program

X(J)	(Z) $X(J) - \mu$	$(X(J) - \mu)^2$
6.000	1.000	1.000
3.000	-2.000	4.000
9.000	4.000	16.000
2.000	-3.000	9.000
4.000	-1.000	1.000
5.000	0.000	0.000
7.000	2.000	4.000
1.000	-4.000	16.000
6.000	1.000	1.000
7.000	2.000	4.000

$\Sigma = 50.000$ $\Sigma = 56.000$

$\mu = \frac{\Sigma}{N} = \frac{50.000}{10} = 5.000$

$\sigma^2 = \frac{56.000}{9} = 6.222$ log 6.222 = 0.79393

$\frac{0.79393}{2} = 0.39697$

0.39697 = log 2.494

$\sigma = 2.494$

On the basis of this calculation, the predicted computer print-out would be of the form shown in Table 3. X's are used to indicate that the longhand calculation was not carried out beyond the indicated number of places. The computer calculation reflects eight figures.

Table 3

Expected Computer Print-out On The Basis Of The Hand Calculation Of The Test Data Of Table 2.

```
        .2494XXXXE+01
        .10000000E+01
        .10000000E+01
        .20000000E+01
        .40000000E+01
        .30000000E+01
        .10000000E+01
        .00000000E+01
        .20000000E+01
        .40000000E+01
        .10000000E+01
        .20000000E+01
AMEAN=          .50000000E+01
B=              .2506XXXXE+01
```

```
C=              .7494XXXXE+01
D=              .11XXXXXXE+01
E=              .9988XXXXE+01
```

The first line of this suggested print-out is the standard deviation (ASIG), which was calculated to be 2.494. The next line is the identification number MM, in this case 1. The next 10 lines are the 10 values of (Z) listed in Table 2. This is then followed by values of AMEAN,B,C,D, and E. The data were then processed and the resulting print-out is shown in Table 4.

Table 4

Actual Computer Print-out Of The Test Data Of Table 2

```
        .24944383E+01
        .10000000E+01
        .10000000E+01
        .20000000E+01
        .40000000E+01
        .30000000E+01
        .10000000E+01
        .00000000E+01
        .20000000E+01
        .40000000E+01
        .10000000E+01
        .20000000E+01
AMEAN=          .50000000E+01
B=              .25055617E+01
C=              .74944383E+01
D=              .11123400E+01
E=              .99888766E+01
```

Comparison of this print-out with that shown in Table 3 suggested that the computer processing of the data was satisfactory, and this method was then used to test the statistical acceptability of the data.

The sets of data in Table 6 are in disintegrations per minute. Each of the twelve lists represents repeated counts of the same vial. The source of the radioactive material in each vial can be determined from Table 5. In each case, a 50λ aliquot of the sample was counted.

Table 5

Identification And Source Scheme For The Data Of Table 6

Identification number	Isotope	Source
401	C^{14}	4 hr urine sample
402	H^3	4 hr urine sample
403	C^{14}	12 hr urine sample
404		12 hr urine sample
405	C^{14}	24 hr urine sample
406	H^3	24 hr urine sample
407	C^{14}	36 hr urine sample
408	H^3	36 hr urine sample
409	C^{14}	concentrated ethyl acetate extract
410	H^3	concentrated ethyl acetate extract
411	C^{14}	concentrated diethyl ether extract
412	H^3	concentrated diethyl ether extract

Table 6

Disintegrations Per Minute Of C^{14} Or H^{3} From The Sources Shown In Table 5

401	402	403	404	405	406
126	126	416	426	507	506
127	121	413	420	519	490
126	126	418	431	526	483
127	117	442	416	518	488
130	119	436	419	528	496
133	123	427	414	530	489
127	120	418	421	517	500
126	120	427	416	511	491
130	124	427	427	520	487
		430	418	512	481

407	408	409	410	411	412
79	77	232	239	211	226
78	77	230	239	197	223
86	89	237	239	206	224
80	81	231	237	207	216
87	83	240	230	215	225
77	76	238	229	208	209
81	81	233	220	221	219
83	80	238	219	214	220
81	77	233	220	221	221
81	76	233	231	212	216

The data were then punched onto computer punch cards in the fashion indicated in Table 7 (each line is a separate card).

Table 7

Manner Of Entering Data Onto Computer Punch Cards. Cf. Table 6. These Data Are Listed Under 401

```
          9        401

126.
127.
```

9 401

126.
127.
130.
133.
127.
126.
130.

The first number entered on the first card is the number of members of this particular set of data, in this case, 9. The next number, also on this card is the identification number MM. These are both read according to FORMAT I10 and, therefore, must be right justified in the 10 and 20 spaces respectively. The next ten cards are the data input, and are read according to FORMAT F10.0. Therefore, it is not necessary to have these right justified, but the location of the decimal point must be indicated.

A similar series of cards was punched for each of the sets of data in Table 6. The cards were then read into the computer and the print-out shown in Table 8 was thus obtained.

Table 8

Computer Print-out Resulting From The Calculations Carried Out On The Data In Table 6

.23333334E+01
.40100000E+03
.22222200E+01
.12222200E+01

```
        .22222200E+01
        .12222200E+01
        .17777800E+01
        .47777800E+01
        .12222200E+01
        .22222200E+01
        .17777800E+01
AMEAN=          .12822222E+03
B=              .12588889E+03
C=              .13055555E+03
D=              .12355555E+03
E=              .13288889E+03
        .31534814E+01
        .40200000E+03
        .42222200E+01
        .77778000E+00
        .42222200E+01
        .47777800E+01
        .27777800E+01
        .12222200E+01
        .22222200E+01
        .17777800E+01
        .17777800E+01
AMEAN=          .12177778E+03
B=              .11862430E+03
C=              .12493126E+03
D=              .11547082E+03
E=              .12808474E+03

        .92400096E+01
        .40300000E+03
        .94000000E+01
        .12400000E+01
        .74000000E+01
        .16600000E+01
        .10600000E+01
        .16000000E+01
        .74000000E+01
        .16000000E+01
        .16000000E+01
        .46000000E+01
AMEAN=          .42540000E+03
B=              .41615999E+03
C=              .43464001E+03
D=              .40691998E+03
E=              .44388002E+03
```

```
        .55136195E+01
        .40400000E+03
        .52000000E+01
        .80000000E+00
        .10200000E+02
        .48000000E+01
        .18000000E+01
        .68000000E+01
        .20000000E+00
        .48000000E+01
        .62000000E+01
        .28000000E+01
AMEAN=          .42080000E+03
B=              .41528638E+03
C=              .42631362E+03
D=              .40977276E+03
E=              .43182724E+03

        .75542483E+01
        .40500000E+03
        .11800000E+01
        .20000000E+00
        .72000000E+01
        .80000000E+00
        .92000000E+01
        .11200000E+02
        .18000000E+01
        .78000000E+01
        .12000000E+01
        .68000000E+01
AMEAN=          .51880000E+03
B=              .51124575E+03
C=              .52635425E+03
D=              .50369150E+03
E=              .53390850E+03

        .76368987E+01
        .40600000E+03
        .14900000E+02
        .11000000E+01
        .81000000E+01
        .31000000E+01
        .49000000E+01
        .21000000E+01
        .89000000E+01
        .10000000E+00
        .41000000E+01
        .10100000E+02
```

```
AMEAN=          .49110000E+03
B=              .48346310E+03
C=              .49873690E+03
D=              .50637380E+03
E=              .47582620E+03

        .32335051E+01
        .40700000E+03
        .23000000E+01
        .33000000E+01
        .47000000E+01
        .13000000E+01
        .57000000E+01
        .43000000E+01
        .30000000E+00
        .17000000E+01
        .30000000E+00
        .30000000E+00
AMEAN=          .81300000E+02
B=              .78067495E+02
C=              .84533505E+02
D=              .74832990E+02
E=              .87767010E+02

        .40838435E+01
        .40800000E+03
        .27000000E+01
        .27000000E+01
        .93000000E+01
        .13000000E+01
        .33000000E+01
        .37000000E+01
        .13000000E+01
        .30000000E+00
        .27000000E+01
        .37000000E+01
AMEAN=          .79700000E+02
B=              .75616157E+02
C=              .83783844E+02
D=              .71532313E+02
E=              .87866787E+02

        .34399612E+01
        .40900000E+03
        .25000000E+01
        .45000000E+01
        .25000000E+01
        .35000000E+01
        .55000000E+01
        .35000000E+01
        .35000000E+01
```

```
        .15000000E+01
        .35000000E+01
        .15000000E+01
        .15000000E+01
AMEAN=          .23450000E+03
B=              .23106004E+03
C=              .23793996E+03
D=              .22762008E+03
E=              .24137992E+03

        .82603470E+01
        .41000000E+03
        .87000000E+01
        .87000000E+01
        .87000000E+01
        .67000000E+01
        .30000000E+00
        .13000000E+01
        .10300000E+02
        .11300000E+02
        .10300000E+02
        .70000000E+00
AMEAN=          .23030000E+03
B=              .22203965E+03
C=              .23856035E+03
D=              .21377931E+03
E=              .24682069E+03

        .72387844E+01
        .41100000E+03
        .20000000E+00
        .14200000E+02
        .52000000E+01
        .42000000E+01
        .38000000E+01
        .32000000E+01
        .98000000E+01
        .80000000E+00
AMEAN=          .21120000E+03
B=              .20396122E+03
C=              .21843878E+03
D=              .19672243E+03
E=              .22567757E+03
```

.51736512E+01
.41200000E+03
.61000000E+01
.31000000E+01
.39000000E+01
.41000000E+01
.51000000E+01
.10900000E+02
.90000000E+00
.10000000E+00
.11000000E+01
.39000000E+01

AMEAN= .22041833E+03
B= .21522469E+03
C= .22599198E+03
D= .21007103E+03
E= .23075562E+03

The value of the mean and the range of $\pm 2\sigma$ were then considered. These values are shown in Table 9 and are plotted as functions of time in Graph #1 and Graph #2.

Table 9

Values Of $\mu \pm 2\sigma$ For The Data Of Table 6

Identification number	Mean	Mean-2σ	Mean+2σ
401	128	124	133
402	122	115	128
403	425	407	444
404	421	410	431
405	519	504	534
406	491	176	506
407	81	75	88
408	80	72	88
409	235	228	241
410	230	214	247
411	211	197	226
412	220	210	230

Next, the value of C^{14}/H^3 was determined. The entries in Table 10 were arrived at in this fashion:

For the 4 hr urine sample:

$$C^{14}/H^3 = \frac{\text{mean } 401}{\text{mean } 402}$$

$$\text{lower limit} = \frac{(\text{mean} - 2\sigma)\ 401}{(\text{mean} + 2\sigma)\ 402}$$

$$\text{upper limit} = \frac{(\text{mean} + 2\sigma)\ 401}{(\text{mean} - 2\sigma)\ 402}$$

Table 10

Range Of Acceptability Of The Mean Of Each Group Of Data

Time	C^{14}/H^3	Lower Limit	Upper Limit
6 hr	1.05	0.970	1.15
12 hr	1.01	0.942	1.08
24 hr	1.05	0.997	1.12
36 hr	1.01	0.850	1.22
ethyl acetate extract	1.02	0.934	1.12
diethyl ether extract	0.960	0.857	1.07

A similar calculation was carried out for the data from each group of rats tested. The results of these calculations are shown in Table 11 and Table 12.

Table 11

Values Of $\mu \pm 2\sigma$ For The Disintegration Rates Of Urine From 5 Groups Of Dosed Rats

Identification number	Mean	Mean - 2σ	Mean + 2σ
101	133	126	139
102	131	122	140
103	420	410	431
104	410	398	422
105	506	490	521
106	504	491	518
107	79	70	89
108	78	73	83
109	222	205	239
110	229	219	239
111	186	173	200
112	186	175	197
201	125	122	127
202	112	109	115
203	394	380	407
204	386	364	407
205	471	451	492
206	450	428	471
207	80	71	89
208	78	69	86
209	224	208	240
210	220	194	246
211	221	208	235
212	223	204	241
301	120	113	127
302	114	106	123
303	397	389	403
304	389	382	396
305	483	477	490
306	455	447	463
307	79	70	88
308	73	65	82
309	201	188	214
310	200	170	230
311	198	192	204
312	194	172	216

Table 11 (cont'd)

Identification number	Mean	Mean - 2σ	Mean + 2σ
501	110	94	126
502	98	94	102
503	346	342	351
504	329	319	340
505	427	415	439
506	402	392	411
507	67	60	74
508	65	58	71
509	176	168	184
510	173	164	182
511	167	163	171
512	163	153	173
601	128	121	134
602	122	108	134
603	408	393	425
604	397	381	414
605	419	408	430
606	412	400	424
607	77	68	85
608	77	73	81
609	224	216	232
610	224	216	232
611	189	175	203
612	189	175	204

Table 12

Range Of Acceptable Limits Of The Data Of Table 11

	Time	C^{14}/H^3	Lower Limit	Upper Limit
Group 1	6 hr	1.01	0.900	1.14
	12 hr	1.02	0.969	1.08
	24 hr	1.00	0.946	1.06
	36 hr	1.01	0.843	1.22
ethyl acetate extract		0.969	0.858	1.01
ether extract		1.00	0.990	1.01

Table 12 (cont'd)

	Time	C^{14}/H^3	Lower Limit	Upper Limit
Group 2	6 hr	1.12	1.06	1.16
	12 hr	1.02	0.945	1.12
	24 hr	1.04	0.955	1.15
	36 hr	1.03	0.826	1.29
	ethyl acetate extract	1.02	0.846	1.24
	ether extract	0.990	0.865	1.15
Group 3	6 hr	1.06	0.920	1.19
	12 hr	1.02	0.975	1.07
	24 hr	1.06	1.03	1.10
	36 hr	1.08	0.854	1.35
	ethyl acetate extract	1.00	0.848	1.26
	ether extract	1.02	0.890	1.18
Group 5	6 hr	1.12	0.923	1.34
	12 hr	1.06	1.01	1.13
	24 hr	1.06	1.01	1.11
	36 hr	1.03	0.845	1.27
	ethyl acetate extract	1.02	0.920	1.09
	ether extract	1.02	0.942	1.11
Group 6	6 hr	1.06	0.912	1.24
	12 hr	1.03	0.950	1.11
	24 hr	1.00	0.946	1.06
	36 hr	1.00	0.840	1.16
	ethyl acetate extract	1.00	0.913	1.09
	ether extract	1.00	0.990	1.01

CHAPTER III

RESULTS AND CONCLUSIONS

This research was undertaken to study the demethylation of trimethylpropylsilane in the rat. The compound was prepared with a double radioactive label (C^{14} and H^3) on methyl groups. This was administered to rats and the urine was collected and examined.

Examination of Graph #3 (page 44) reveals that the C^{14}/H^3 ratio remains unchanged at 1.00. The probability is better than 95% that the ratio does not get as high as 1.50. It is within the limits of statistical acceptability that the ratio in the urine is 1.125 and that of the extract 1.00.

Therefore, according to the premises stated in the introduction (page 3), decarboxylation does not occur *in vitro*.

The statistical limits allow for the possibility of a partial oxidation to the hydroxymethyl function *in vivo*, followed by the loss of this functionality upon extraction. However, it should be noted that this is an unlikely sequence, since it has been shown that hydroxymethylsilanes are stable under these extraction conditions.[5]

A further possibility is that demethylation occurs

by direct methyl transfer. This route is known to occur with N-methyl groups.[9] If this pathway is in operation, the isotopic ratio would not change.

It was presumed, therefore, that demethylation was occurring *in vivo*, probably by a direct methyl transfer.

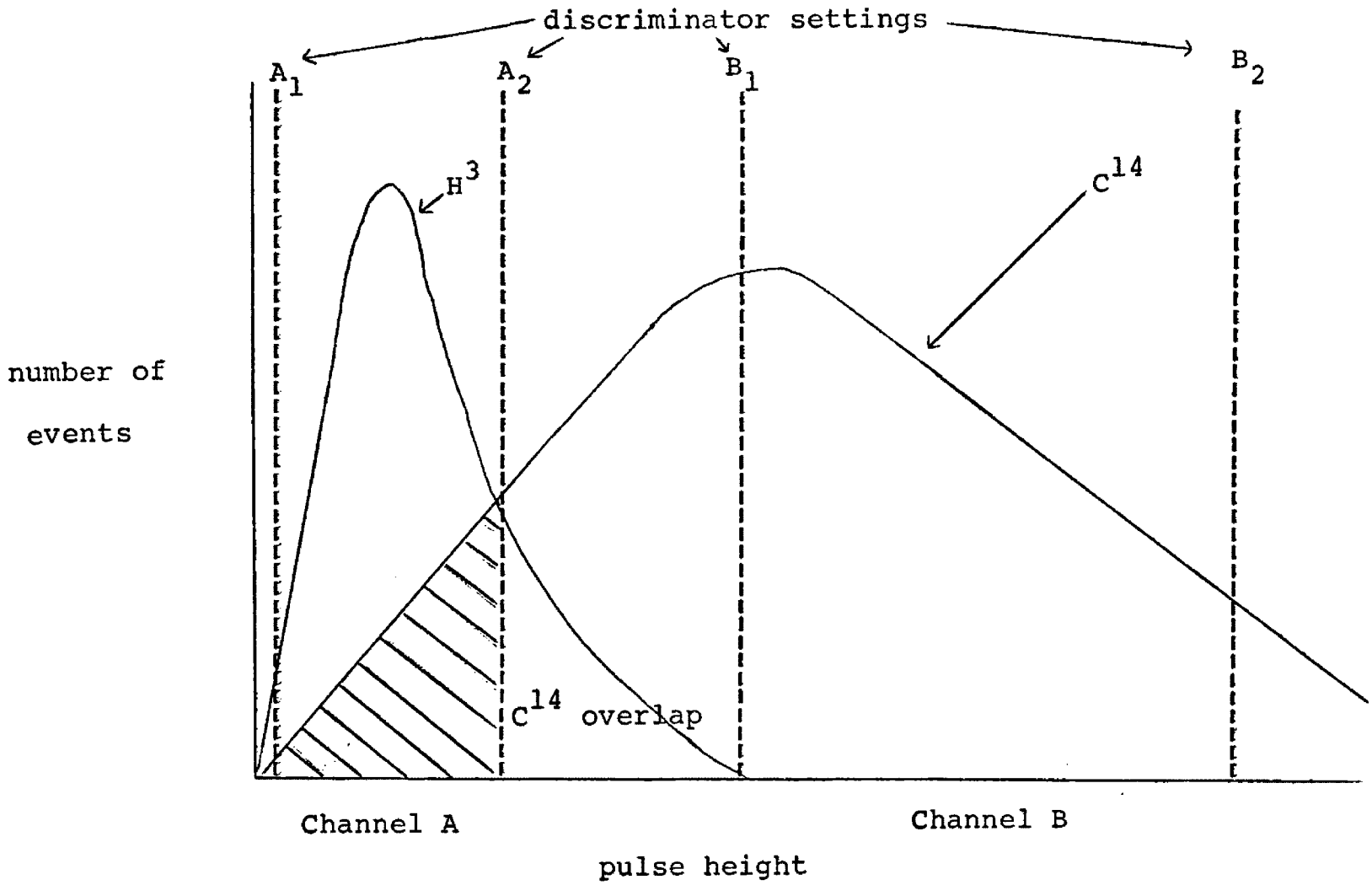


Fig. 1

number of events vs. pulse height, from Chicago Nuclear "Operator's Manual"

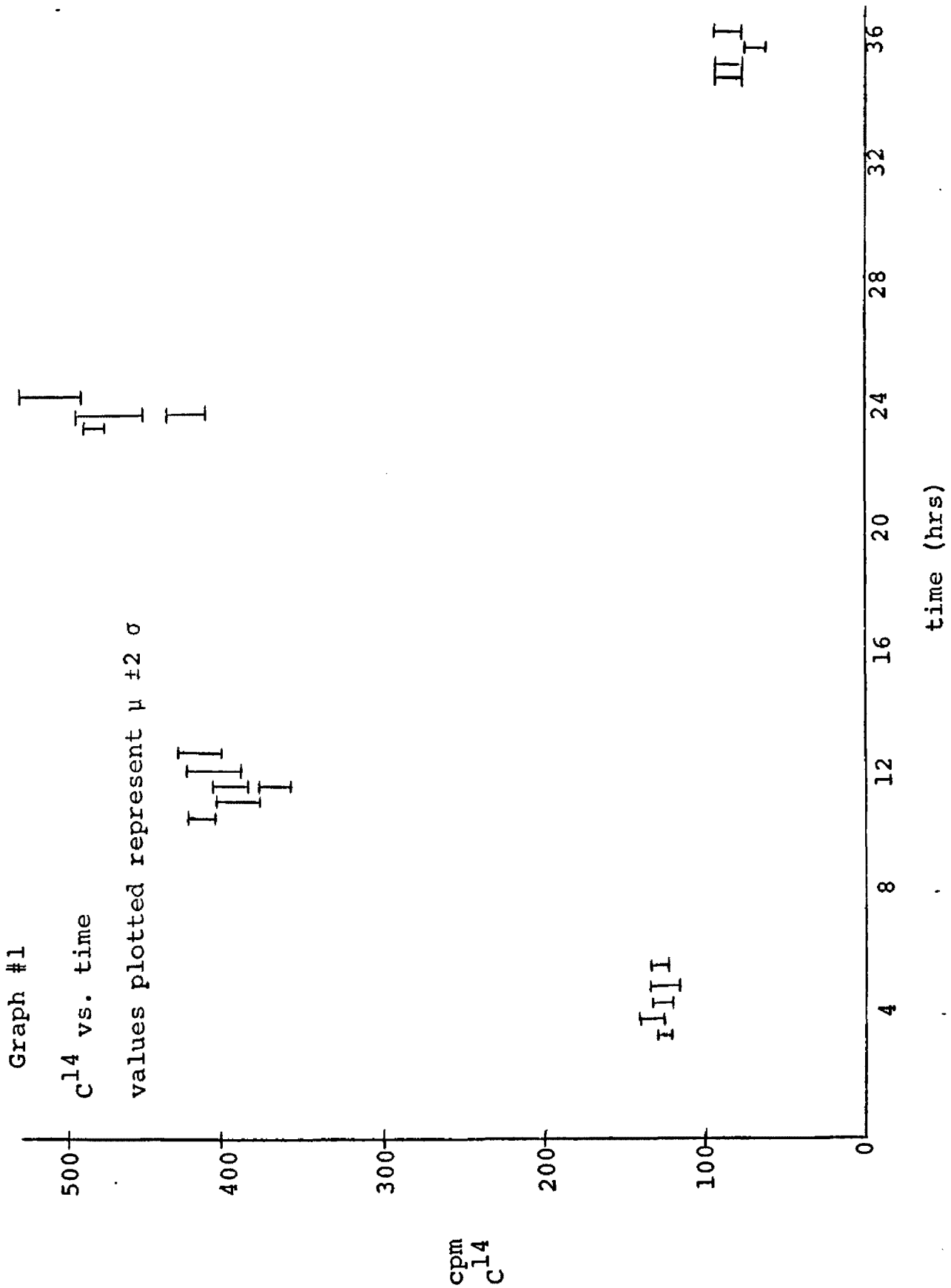
APPENDIX

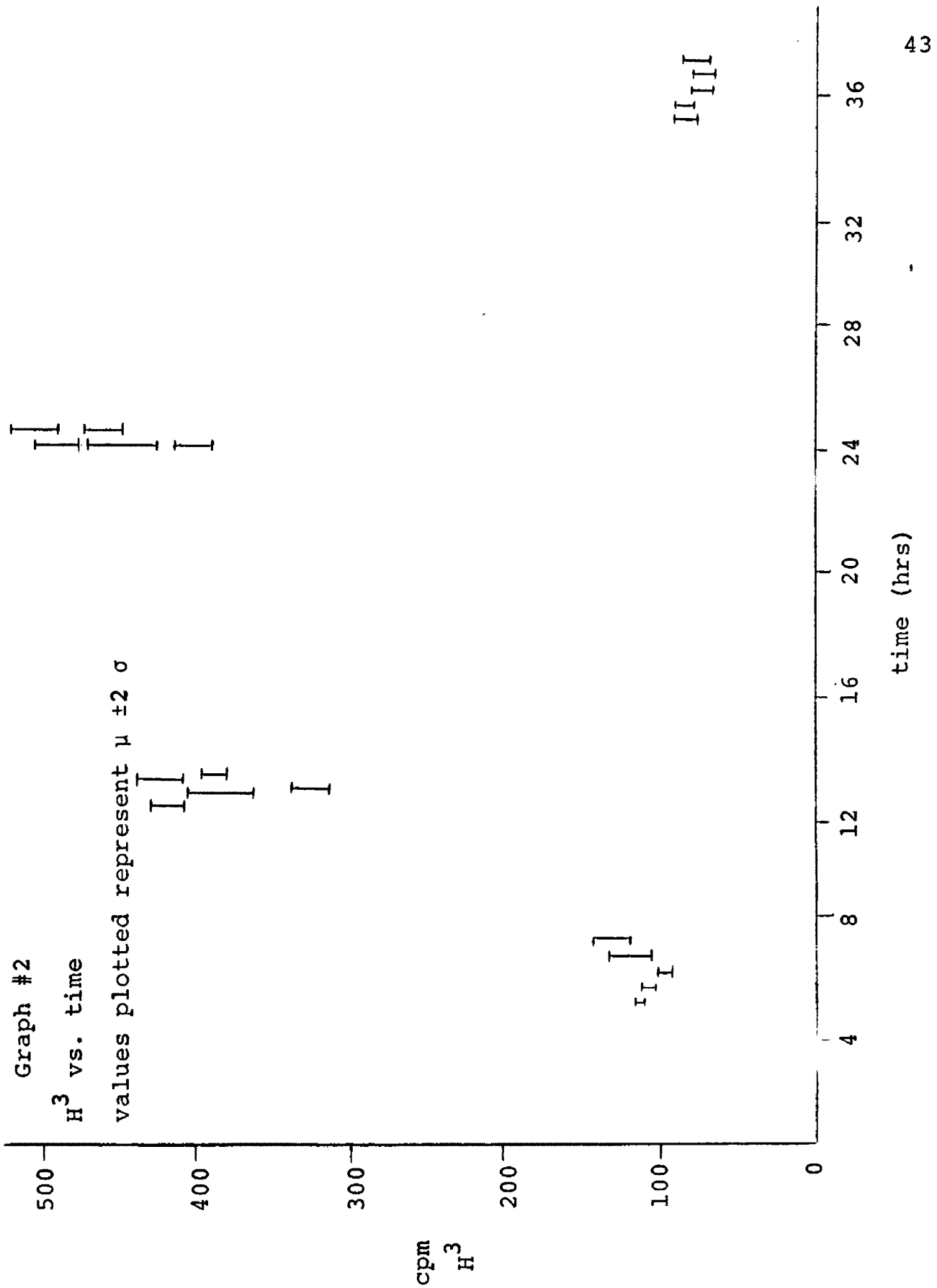
GRAPHS

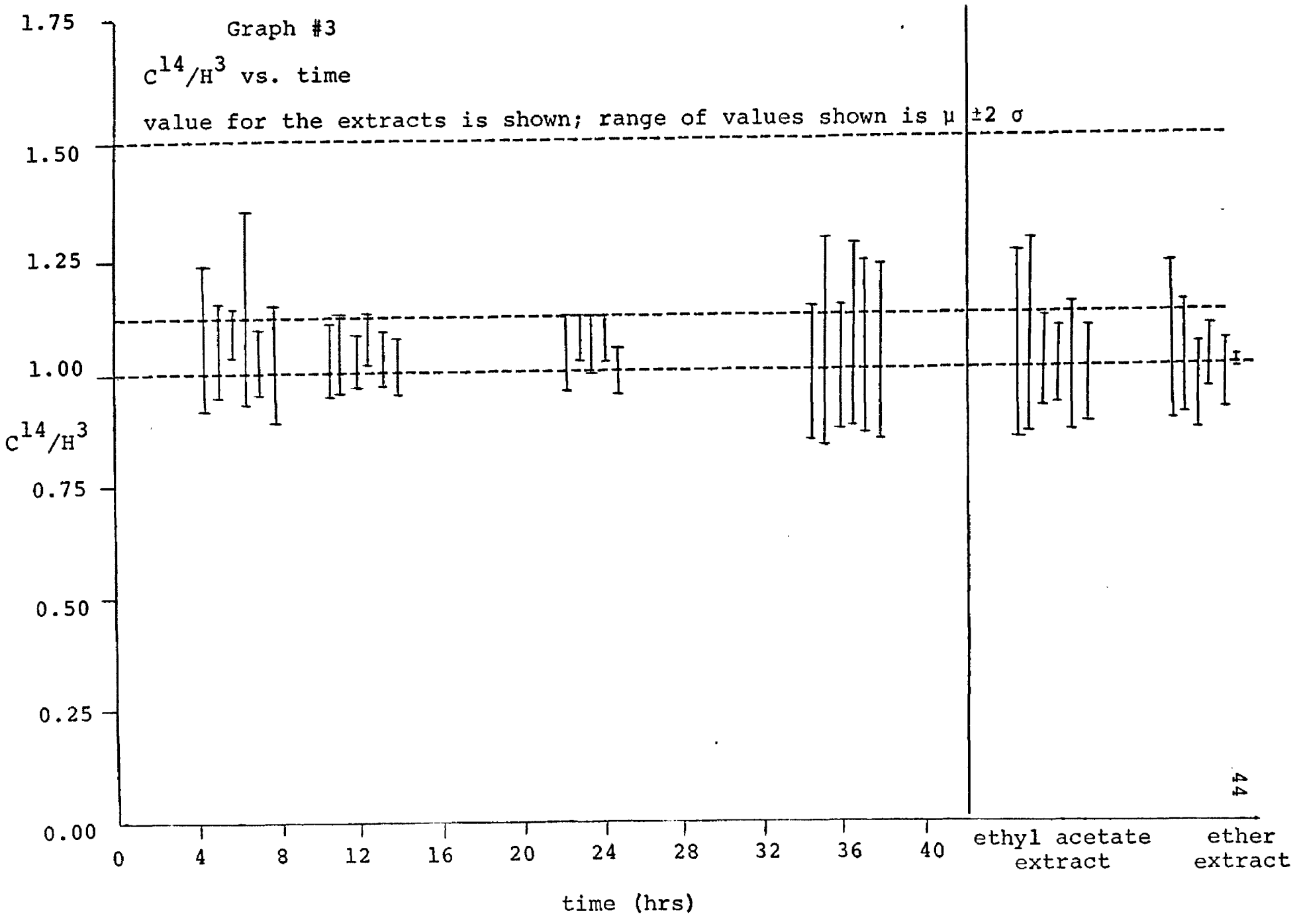
GRAPH #1 .....C^{14} vs. time

GRAPH #2 .....H^{3} vs. time

GRAPH #3 .....C^{14}/H^{3} vs. time







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