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Comparison of Phenyl Ethylamine and Hyamine Base as an Absorbent of Carbon[14] Dioxide

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$C^{14}O_2$ 의 吸收劑로서 페닐에칠아민과 하이아민베이스의 比較

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摘 要

CO_2 의 吸收能力을 比較하기 爲하여 濕潤燃燒實驗에 있어서 Phenyl Ethylamine 과 Hyamine Base 를 使用하였다. 알려지고 있는 肝藏脂質과 均一하게 混合된 팔미틴산—C^{14} 을 硫酸과 燐酸으로 酸化하였다. 生成된 CO_2 를 가느다란 비닐管을 통하여 供試아민劑에 捕捉시켰다. CO_2 를 吸收한 아민 劑들을 Diotal Scintillator와 混合하고 Liquid Scintillation Counter 에 依하여 그 放射能을 測定하였다. 供試한 2種아민의 計測効率은 50～60% 이 었으며 放射能回收率은 거의 100% 이었다. 그러나 Phenyl Ethylamine 에 依한 CO_2 吸收量이 Hyamine Base 의 그것에 比하여 約 2倍가 되었으며 取扱하기도 容易하고 代金도 싸기 때문에 CO_2 吸收劑로서는 Phenyl Ethylamine 을 使用함이 좋을 것이다.

한편 돼지의 肝藏實驗에 있어서 脂質을 CO_2 로 變化시켜 C^{14} 의 放射能을 測定하는 것이 脂質을 그대로 計測하는 것보다. 計測效率이 더 增加되지 않음이 밝혀졌다.

Introduction

Van Slyke et al. (1951)[12] and Steele et al. (1956)[11] measured the total carbon content with a manometer after absorption of carbon dioxide to the sodium hydroxide solution, and measured the radioactivity of carbon[14] dioxide in a gas phase using proportional counter. Recently, liquid scintillation counting, which was founded by Reynolds and Kellmann and their associates independently in 1950, became more useful with β-emitting isotope because of its high efficiency and relative freedom from loss of activity by self-absorption (Bell and Hayes, 1958).[1] However, the composition of the solvent which may be used practically in scintillation systems is restricted to a small number of organic compounds. Toluene appeared to be the preferred solvent, but there are many substances it cannot dissolve.

It had been found that carbon dioxide could be dissolved in toluene by combinating it with a high molecular weight quarternary ammonium hydroxide (Hyamine base, Passmann et al. 1956)[9], Bray (1968)[2] and Bray and Goodman(1968)[3] measured the radioactivity of carbon[14] dioxide from organic acid C^{14} and glucose-C^{14} with this amine as an absorbent of that gas. On the other hand more effective and convenient absorbent have been studied. woeller(1961)[13] experimented with several primary amines including Hyamine base and phenyl ethylamine in order to cover an appreciable quenching and high cost of Hyamine base. Kelly et al. (1961)[8] studied with tertiary alkyl primary amine which did not absorb quantitatively the carbon[14] dioxide. Woeller (1961)[13] published that phenyl ethylamine (or phenethylamine) showed most effective results.

However, Woeller used $BaC^{14}O_3$ as a source of carbon dioxide. Here in this experiment the lipid of swine was used, which had yellow colour in toluene scintillator and brought appreciable quenching (Chiang, 1969)[5].

Main purpose of this experiment was a comparison between Hyamine base and phenyl ethylamine for quantitative absorption of carbon[14] dioxide from organic materials. But the result of this experiment could be used for the trapping of carbon[14] dioxide from various inorganic carbonate more easily. By the way, this technique might be used for the respiration experiments of animals directly for the expired air or via carbonate described above.

At the same time this oxidation method was compared to the direct counting of radioactivity in liver lipid of swine.

Experimental Procedure.

1. Chemicals.

Hyamine base: Hyamine chloride solution in toluene and methanol was treated with Ag_2O, and Hyamine hydroxide (base) solution manufactured was exposed to the sunlight for about one month. The precipitated and suspended materials in the solution was discarded by centrifugation. Final concentration of the supernatant was 1 M in methanol (Eisenberg, 1958)[6].

2-Phenyl ethylamine was laboratory reagent grade manufactured at British Drug House Ltd.

Digestion acid was made by mixing 600 ml of concentrated sulfuric acid and 400 ml of 85 per cent phosphoric acid, and potassium dichromate was used as a catalyst (Shaw, 1959)[10].

Dioxane scintillator (NE 220-II: PPO 6g, dimethyl POPOP 0.6g, naphthalene 50 g and p-dioxane 1000 ml) and diotal scintillator (PPO 6.5g, POPOP 0.13g, naphthalene 104g, dioxane 500 ml, toluene 500 ml and methanol 300 ml) were cmpared for the Hyamine solution counting. Dioxane scintillator provided some precipitation and unclear solution, but the latter brought no problems. It was thought that Hyamine carbonate present after digestion might be partly insoluble in dioxane scintillator but soluble in methanol and toluene. Passmann (1956)[9] and Eisenberg (1958)[6] used toluene scintillator when inorganic carbonate were employed as a carbon source. Since organic material and water was placed in a flask in this experiment, dioxane and naphthalene had to be used. According to this preliminary trial it was shown that methanol contributed to make one phase system. Woeller (1961)[13] also studied on solvents for phenyl ethylamine, phenyl ethylammonium phenethyl carbamate present after trapping CO_2 was very soluble in alcohol (methanol) but much less so in toluene. His preparation was that 27 ml of redistilled phenthylamine, 27 ml of absolute methanol, 500 mg PPO and 10mg POPOP was mixed and diluted with toluene to 100 ml. This composition is very similar to the diotal scintillator except phanyl ethylamine. Thus, this amine and diotal scintillator(1 : 3 in volume) were mixed in long thin tube to trap quantitatively CO_2.

2. Apparatus.

250 ml Erlenmeyer flask was used for the digestion of samples, carbon dioxide produced was introduced to the absorbent through polyethylene tube (1.2 mm in diameter, 0.1 mm in thickness, for 3 ml of 1 M Hyamine base methanol solution) and through glass tube (1.2 mm in diameter, 1.2 mm in thickness, for 6 ml of 1 : 3 phenyl ethylamine scintillator solution), respectively.

Since the volume of the absorbent was little, thin glass tubes containar was used: 4.5 mm and 9.0 mm in diameter, respectively.

In Hyamine experiment, hand pressing rubber bulb was used for air suuply, while piston type small compressor was attached to the digestion apparatus in phenyl ethylamine study.

CO2 free air was provided by means of 30% potassium hydroxide.

3. Digestion procedure.

Total lipid in swine liver was isolated by the method of Folch(1957). Proper amount of lipid was taken from petroleum solution of liver lipid, which involved known activity of palmitic acid-C^{14}. The petroleum ether was evaporated in an oven under nitrogen gas to prevent any oxidation of lipid at 50°C.

Two ml of potassium dichromate and two ml of water were added. Immediately after addition of digestion acid (15ml), rubber stopper was capped tightly and heated gently. Very little bubbles in diameter were occured and run through the thin tube.

At Hyamine experiment the middle part of polyethylene tube was placed in a cold water to avoid hot gas. Eisenberg (1958)(6) said that Hyamine base was thermolabile. Occasional air-suuply was conducted through the rubber compressor.

The cooling system was cancelled and regular air-supply (but in little amount of air) was carried out by a motor compressor in phenyl ethylamine study.

Carbon dioxide-absorbed solution was then mixed with diotal scintillator in glass vial after complete oxidation of lipid sample by heating vigorously, gradually increased the flame. Beckman Liquid Scintillation System was employed for the counting of radioactivity in sample solution which had been placed in dark room overnight. An internal standardization method was used for getting counting efficiency.

Results.

1. Hyamine bases amount.

Preceding the digestion experiment the relationship between the amount of Hyamine base and scintillator volume was studied, not using carbon dioxide. Brown (1961)(4) used 1.15ml of 1 M Hyamine base methanol solution and 4 ml of NE 213 scintillator to count the activity of 0.1 ml of glycine-C^{14} water solution. Eisenberg (1958)(6) employed 2ml of 1 M base solution trapped carbon dioxide, and 14 ml of toluene scintillator. Passmann (1956)(9) mixed 2ml of 1M base with 35ml toluene.

For 10 ml of diotal scintillator, 1.0 to 3.0 of 1M Hyamine base methanol solution was added in interval of 0.5 ml as in table 1. Then 50 μl of U-C^{14}-glucose dioxane solution (20.979 dpm) was added as a standard to each vial. Maximum count rate was investigated according to the gains in interval of 50, and 750 gain was found. Back count rate was subtracted from samples count rate. Finally, counting efficiency was calculated (Table 1) 1.0 ml of Hyamine base showed best result, and it was known that the less the amount of the base, the more the efficiency. Passmann (1956)(9), before decision of 2ml base with 35ml scintillator as mentioned above, 0.47 to 4.63 ml of base was added to the 35ml of phosphor and compared the counting efficiencies. 1.4ml of base showed highest efficiencies, however, he adopted 2ml because of its good recovery. 2ml of base with 35ml phosphor corresponds to 0.4ml base for 10 ml.

This amount of base is very little and not practicable, therefore, dilution or division of absorbed solution was made after digestion of lipid in this experiment.

Table. 1. Counting efficiency of solutions contaning various amount of Hyamine base.

Hyamine base(ml.)	Count per min.	CPM corrected	Theoretical DPM	Efficiency (%)
1.0	12.441	12.421	20.979	59.2
1.5	11.896	11.874	20.979	56.6
2.0	10.989	10.965	20.979	52.3
2.5	10.286	10.260	20.979	48.9
3.0	9.522	9.494	20.979	45.3

2. Recovery of activity through Hyamine base.

Passmann (1956) and Eisenberg (1958) recommended 1.1 mmole of Hyamine base for 1 mmole of carbon dioxide. Total lipid content in 1 g of swine liver, which amount of sample was advised by Folch (1957)[7], is about 50 mg. This amount corresponed to 3 mmoles of CO_2, thus, about 3.3 mmoles of Hyamine base solution would be necessary.

However, placing 30, 40 and 50 mg of lipid in digestion flask, 3ml of convenient amount of Hyamine base was pipetted in glass tube which was closed in bottom end. After about one hour of heating the carbonated solution was poured in counting vial and the glass tube was rinsed with diotal scintillator twice. The whole amount of scintillator used for rinsing and diluting was 20 ml and half the solution was poured into another vial. That is, 1.5ml of Hyamine base was mixed with 10 ml. This amount of base is more than Passmann's 0.4 ml, but is less than Brown's 2.9 ml and similar to Eisenberg's 1.4ml.

Table 2. Radioactivity recovery through Hyamine base.

Lipid (mg)	Samlpe No.	Count per min.	CPM corrected	Efficiency (%)	DPM of sample	Total DPM	Recovery (%)
30	1	9.163	9.135		16.430		
	1+st+)	20.818	11.655	55.6			
	2	10.007	9.979		18.514	34.944	100.7
	2+st	21.314	11.307	53.9			
40	1	10.081	10.053		17.856		
	1+st	21.894	11.813	56.3			
	2	11.195	11.167		20.490	38.346	97.6
	2+st	22.629	11.434	54.5			
50	1	12.399	12.371		21.589		
	1+st	24.435	12.036	57.3			
	2	11.242	11.214		19.007	40.596	92.6
	2+st	23.626	12.384	59.0			

+) st: 50μl of C^{14} –palmitic acid (22.866 DPM).

Viewing from table 2 it was known that 3.0 ml(3.0 mmole) of Hyamine could absorb carbon dioxide from 30 mg or 40 mg lipid, but some insufficient for 50 mg. This result confirmed the publishment by Passmann (1956) and Eisenberg (1958). They recommended 3.3 mmole of base for 3 mmole of carbon dioxide.

But, by the way, it was recognized that about 1.5 ml of Hyamine base could be used for 10 ml of diotal scintillator. This level is about 4 times that by passmann, but similar to that by Eisenberg.

3. The amount of phenyl ethylamine.

Woeller (1961)[13] published 1.5 ml of phenyl ethylamine-alcohol-toluene mixture for quantitative trapping one mmole of carbon dioxide. This mixture contains 0.405 ml of phenyl ethylamine. And he recommended 7.5 ml of phenethylamine mixture being mixed in 10 ml of final volume with diotal scintillator. This mixture contains 2.025 ml of phenethylamine. In this trial 1.0 to 3.0 ml of phenethylamine was mixed with 10 ml of diotal scintillator in 0.5 ml interval (Table 3).

Although much amount of normal air was introduced into these vials for about 30 minutes to prepare similar condition as in real experiment, the counting efficiencies were very high; most of samples brought more than 60% efficiencies. Among them 1.5 ml showed highest value (66

Table 3. Counting efficiency of solutions containing various amounts of phenyl ethylamine.

Phenethylamine (ml)	Count per min.	CPM corrected	Theoretical DPM	Efficiency (%)
1.0	10.067	10.042	15.607	64.3
1.5	10.344	10.319	15.607	66.1
2.0	9.530	9.505	15.607	60.9
2.5	9.512	9.487	15.607	60.8
3.0	9.411	9.386	15.609	60.1

%).

4. Recovery of activity through phenyl ethylamine.

1.5ml of phenylethylamine could me mixed with 4.5 ml of diotal scintillator and this 6 ml of mixture could absorb about 4 mmole of carbon dioxide, which amount correspond to 67 mg of lipid, according to Woeller (1961)[13].

But only 50 mg of lipid was placed in the digestion flask and carbon dioxide produced was trapped by 6.0 ml of phenethylamine diotal mixture (Table 4).

Table 4. Radioactivity recovery through phenyl ethylamine.

Sample No.	Count per minute	CPM corrected	Efficiency (%)	DPM from sample	Recovery (%)
1	11.891	11.866		21.575	98.3
1+st+)	20.470	8.587	55.0		
2	12.513	12.488		23.169	107.3
2+st	20.927	8.414	53.9		
3	11.586	11.561		22.624	104.8
3+st	19.557	7.971	51.1		
Mean			53.3		103.5

+) st; 50μl of u-C^{14} glucose in diotal solution (15.607 DPM).

Average recovery was 103.5%. It is interesting to view that the efficiency of these samples are much lower than those from samples in preliminary experiment, that is, various quenching substances other than phenethylammonium phenetylcarbamate has been produced. Clearly different thing is that some water vapor has been involved in this solution.

5. Direct using of lipid sample for scintillation counting.

Chiang (1969)[5] experimented on counting techniques using direct lipid and C^{14}-palmitic acid added to toluen scintillator. At gain 900, 50 mg lipid sample gave 58% efficiency and 108% recovery. Table 5 shows that same amount of lipid brough same efficiency and 101% recovery at maximum gain of 850 in this experiment.

Table 5. Radioactivity recovery when lipid was directly counted with toluene scintillator

Gain	Lipid (mg)	Count per min.	CPM corrected	Efficiency (%)	DPM from sample	Recovery (%)
800	50	7.340	7.250		11.964	96.1
	50+st+)	21.186	13.846	60.6		
850	50	7.375	7.289		12.567	101.0

	50+st	20.639	13.264	58.0		
900	50	6.317	6.237		11.059	92.5
	50+st	19.205	12.888	56.4		

+) st; 50 μl of C^{14}-palmitic acid toluene solution (=22.866 DPM).

Discussion.

1. Hyamine base.

It is reasonable to view that the less amount of Hyamine base shows more efficiency on table 1. Following the publishment of Passmann (1956)[9] 0.4 ml would show maximum efficiency. However, it would be very difficult and practically impossible to use so little amount of base directly for the quantitative absorption. Hence, if more amount of Hyamine base is used the CO_2-absorbed solution should be diluted further and divided into several vials, This is most disadvantageous point especially in most of biological experiments where the trace amount of radioisotope is advised to be used.

Second point is that it is laborous to prepare stable reagent and takes long time. And there is still another shortcoming; it is expensive. The fourth one is the difficulty of handling because of its viscosity.

2. Phenyl ethylamine.

Although efficiencies of counting were not high, the radioactivity recoveries were good (averagely 103.5%). Woeller (1961)[13] published 60% of counting efficiency from phenyl ethylamine counting, which absorbed 2 mmole of $carbon^{14}$ dioxide from carbonate source. This more efficiency than 53% present here (table 4) might be derived from non-water system. The carbon dioxide gas would be liberated from carbonate without occurence of any water vapor. However, 2 ml of water was added to the digestion flask to promote the reaction (Shaw, 1959)[10] and some more water vapor would be produced from oxidation of lipid in this study.

Only 1.5 ml of phenyl ethylamine was used as the absorbent of CO_2 because of the highest efficiency among tested (table 3) and of unnecessity to use more absorbent for 50 mg lipid. Woeller (1961) stated that, in a custom-built spectrometer operating in coincidence at room temperature, 2.0 ml of phenyl ethylamine could absorb quantitatively the CO_2 gas (5 mmole) and was counted at 50% efficiency, the final volume was 10 ml in each vial.

Eisenberg (1958)[6] recommended 1.1 mmole of carbon dioxide for final volume of 10 ml solution containing 1.2 ml of Hyamine base and 8.8 ml of toluene scintillator. Applying 1.2 mmole of CO_2 to 10 ml of final volume, quantitative trapping of the gas has been experienced in this experiment with Hyamine base.

It is reasonable to say that about 2 times carbon dioxide could be absorbed into each vial involving phenyl ethylamine compared to the hyamine base. However, 4.5 times could be employed according to the procedure by Woeller (1961)[13]. The large capacity would be heltpful in many biological experiment.

Furthermore, it is not necessary to prepare special reagent, i.e., phenyl ethylamine could be used directly. It is not expensive and less viscous than Hyamine base reagent.

This procedure could be employed in many fields of biological studies, for example, overall counting of various tissues, coloured samples or chemically quenching materials. And carbon dioxide from respiration experiments could be absorbed to phenyl ethylamine directly or via carbonates.

3. Comparison with direct counting technique.

Total lipid of swine liver showed yellow color in toluene scintillator and brought some much quenching (58% counting efficiency for 50 mg lipid) and some inacurate counting result (108% recovery, Chiang 1969)[5].

This experiment had a purpose in part to avoid this quenching and inacuracy. But Hyamine base and phenyl ethylamine brought also similar result in this trial. Therefore, concerning total lipid of swine liver, this oxidation tenchique could not be useful. Restudy on direct counting of liver lipid showed 58% efficiency and 101% recovery (Table 5).

But other compositions or samples such as proteins, tissues, sodium carbonate, barium carbo nate could be oxidized or converted to the CO_2 and absorbed to phenethylamine.

Summary.

In order to compare their absorption abilitites of carbon dioxide, Hyamine base and phenyl ethylamine were employed in wet combustion experiments. The known content of liver lipid and uniformly mixed palmitic acid-C^{14} were oxidized with sulfuric acid and phosphoric acid. Carbon dioxide produced was trapped by the amines tested through thin tube. The amines absorbed carbon dioxide were mixed with diotal scintillator and counted by liquid scintillation counter.

The counting efficiencies and radioactivity re-coveries were 50 to 60 % and nearly 100 % for both amines. However, the absorption ability of carbon dioxide by phenyl ethylamine was about two times that of Hyamine base.

This technique would not be necessary for the counting of liver lipid of swine, because the effic-iencies were not increased and counting accuracies were similar to those of direct counting.

References

1. Bell, C.G. Jr. and F.N. Hayes, 1958. Liquid scintillation counting. Pergamon Prss Ltd., vii-viii.
2. Bray, G.A. 1968. Lipogenesis from glucose and pyruvate in fat cells from genetically obese rats. J. Lipid. Res. 9 : 681—686.
3. Bray, G.A. and H.M. Goodman, 1968. Effect of epinephrine on glucose transport and metabolism in adipose tissue of normal and hypothyroid rats. J. Lipid Res. 9 : 714—719.
4. Brown, W.D. and H.G. Bodman, 1961. Liquid-scintillation counting of C^{14} labelled animal tissues at high efficiency. Biochem. J. 78 : 571—578.
5. Chiang, Y.H. 1969. Quenching of C^{14} palmitic acid activity by lipid color of swine liver. Report to the I.A.E.A., 1—9.
6. Eisenberg, F. Jr. 1958. Preparation of the alkaline absorbent for radioactive CO_2 in liquid scintillation counting: Bell, C.G. Jr. and F.N. Hayes, 1958. Liquid scintillation counting. Pergamon Press Ltd. pp. 123-125.
7. Folch, J., M. Lees and G.H. Sloanestanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226 : 497-509.
8. Kelly, R.G. et al. 1961. Determination of C^{14} and H^3 in biological samples by Schöniger combustion and liquid scintillation techniques. Anal. Biochem. 2 : 226.
9. Passmann, J.M., N.S. Radin and A. D. Cooper. 1956. Liquid scintillation technique for measuring carbon-14-dioxide activity. Anal. Chem. 28 : 484-486.
10. Shaw, K. 1959. Determination of organic carbon in soil and plant material. J. Soil Sci. 10 : 316-326.
11. Steele, R., J.S. Wall, R.C. de Bodo and N. Altszukr. 1956. Measurement of size and turnover rate of body glucose by the isotope dilution method. Am. J. Physiol. 187 : 15-24.
12. Van Slyke, D.D., R. Steele and J. Plazin, 1951. Determination of total carbon and its radioactivity. J. Biol. Chem. 192 : 769-805.
13. Woeller, F.H. 1961. Liquid scintillation counting of $C^{14}O_2$ with phenethylamine. Anal. Biochem. 2 : 508-511.