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A Rapid Method for the Measurement of the Absolute Activity of Carbon-14 in Pea Plant Tissue

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SUMMARY

A rapid method for the measurement of the absolute activity of carbon-14 in cotyledons and root of etiolated pea seedlings has been developed. Fresh tissue was frozen in liquid air, ground and suspended in gel phosphor and subjected to measurement for its radioactivity by liquid scintillation counter. Apparent activity of the suspended tissue sample calculated by counting efficiency value obtained by internal standardisation, was found to be related to absolute activity of the tissue, determined by flask combustion technique, by a constant factor. Once this factor is determined experimentally, analysis of C-14 lebelled tissue involves only fairly simple suspension counting by liquid scintillation counter. Present method appears to be applicable to other plant tissues tagged with C-14.

INTRODUCTION

In spite of the wide use of carbon-14 as a tracer in many biological experiments, development of a rapid and covenient method of measuring the absolute activity of the isotope in biological tissue has not been reported. Existing methods generally involve the initial combustion of active samples followed by activity measurements either on gaseous carbon dioxide or on a derived carbonate[(1)]. Apart from the elaborate apparatus required for most combustion techniques, this method is also rather tedious and time consuming for routine use.

In a metabolic study of labelled indole-3-acetic acid (C-14) in etiolated pea plants[(2)], it was necessary to determine absolute activities in a large number of root and cotyledon tissue samples. This requirement led to the development of a routine method for measurement of C-14 activity based on scintillation counting of tissue suspensions in gelled liquid scientillators.

For the more usual liquid scientillation technique where active sample and liquid scintillator are in one phase, absolute activities may be calculated from measured count rates using values for counting efficiency determined by the internal standard, channel ratio[(3)], or external standard[(4)] techniques. With suspension counting, the determination of efficiency is less straightforward,

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because in addition to the usual factors affecting efficiency, there are the scattering effects of the suspended materials on light transmission and self-absorption of the beta radiation within the suspendend particles.

We have found that an adequate correction for the effect of the suspension on light collection can be made by the use of an internal standard. The residual effect of self-absorption was then found to be almost constant for all tissue samples investigated and to be related to the absolute activity by a constant factor.

These observations provide the basis of the method which requires a measurement of count-rate for each suspended sample, followed by determination of apparent efficiency by internal standardisation. Absolute activities may then be calculated from a correction factor previously determined by a flask combustion technique for the specific type of tissue. Once determined, this factor may be used for all further samples, and routine assays then require only relatively simple scintillation measurements.

METHODS AND MATERIAL

Preparation of plant meterial

Each plant tissue sample (roots or cotyledons) was prepared by freezing it briefly in liquid air and then transferring it with 50 ml of liquid air to a small mortar. When all the air had evaporated the frozen tissue was immediately ground to a fine powder which, while still frozen, was quantitatively transferred to a weighing bottle and quickly weighed.

For suspension scintillation counting, a portion was transferred with a cooled spatula to a weighed counting bottle and after reweighing, the required volume of a gel phosphor was added.

Flask combustion

Absolute activities were determined by scintillation counting of the carbon dioxide (C-14) obtained by flask combution of the tissue. Many variations of the technique have been described [5,6] and after trial experiments the following procedure was adopted for this investigation.

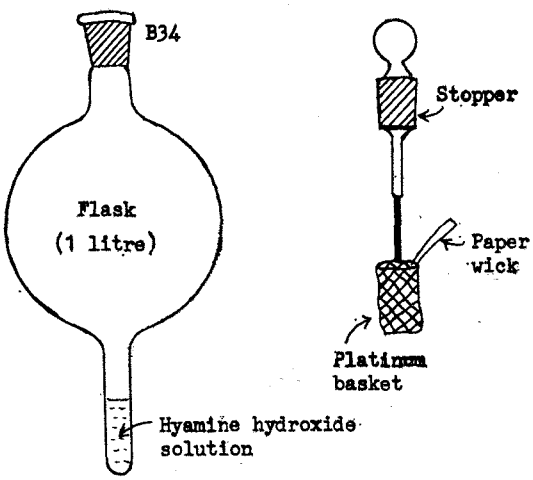

Fig 1. Flask combustion apparatus

The samples of powdered, frozen tissue was freeze-dried and an accurately weighed aliquot (about 90 mg) was wrapped in a piece of filter paper of known weight(about 10 mg) and subjected to combustion in a 1 litre glass flask as shown in Fig. 1. The radioactive tissue, together with a paper wick, was placed in a platinumb asket. Using a long stemmed pipette, a measured volume of Hyamine hydroxide solution* and 5 ml toluene were added to the flask which was continuously cooled by immersion in a dry ice-acetone mixture. The flask was flushed with oxygen for two minutes; combustion was initiated by igniting the paper wick attached to the platinum basket which was then quickly inserted into the flask, taking care to insert the stopper before the sample itself commenced to burn. Combustion lasted for 4-8 seconds. The platinum basket was allowed to cool and then the flask was removed from the dry ice acetone mixture and left to stand for 15 minutes with occasional shaking. The inside wall of the flask was washed down in turn with 4 ml each of toluene (Analar grade) and ethanol (Analar grade) and then the contents of the flask were transferred quantitatively with a pipette to a 25 ml volumetric flask. Any remaining traces of the

*1 molar methanolic p-(diisobutylcresoxyethoxyethyl), dimethylbenzylammonium hydroxide

solution in the combustion flask and platinum basket were removed by washing with small portions of toluene which were then added to the volumetric flask. After making up to 25 ml with toluene, aliquots of this solution were counted by the method described below.

Counting techniques

A coincidence type scintillation counter(Isotope Development Ltd., Type 6012) was used for all measurements of activity. Optimum working conditions were determined using the statistical criterion, S^2/B, discussed by Outtridge [7]. Under the selected optimum settings (EHT 1300 and 1400 V, upper gate 100 mV), the counting efficiency of n-hexadecane-C14 (RCC, Amersham) in PPP-3 phosphor (see below), was 85% with a background of 3.3 cps. This fell to 50%, with 2.1 cps background, when using an upper gate bias of 40 mV as required for measurement of plant material.

For homogeneous counting, a toluene based phosphor(PPP-3) of composition; PPO 3g, POPOP 0.1 g dissolved in 200 ml of toluene(Analar grade), was used throughout. Suspension counting was carried out in a mixed solvent gel phosphor (G/TED)* of the following composition; PPO 6 g, POPOP 0.1 g, naphthalene 40 g, toluene 417 ml, ethanol 333 ml, dioxane 250 ml and silica powder (Cab-O-Sil).** Ethanol and dioxane solvents were purified and dried by normal chemical methods.

In general, 20 ml glass sample bottles sealed with polythene caps were used for counting. For homogeneous counting of Hyamine carbonate, 8 ml of sample were mixed with 2 ml of PPP-3 phoshpor. In suspension counting of plant tissue, 10 ml of gel liquid phosphor (G/TED) was added to the weighed powdered tissue contained in the 20 ml counting bottle and the mixture was vigorously shaken to give an even suspension. To reduce the effect of chemiluminescence,the bottle was stored in a refrigerator for 48 hours and after re-shaking the sample was inserted into the counter. After this dark adaptation period, the chance coincidence count rate due to chemiluminescence was very small in all samples. The small residual rate appeared to be roughly proportional to the amount of fresh tissue suspened.

Wherever possible at least 10,000 counts were obtained giving a coefficient of variation better than 1% for all count rate measurements. Paralysis time losses were negligible but all rates were corrected for background and, where appropriate, for chance coincidence rates. Counting efficiency was determined by internal standardisation using n-hexadecane-C14 introduced into the sample from a weight pipette. For homogeneous scintillation samples from the flask combustion technique, the counting efficiency was checked by the "channels ratio" method developed by Baillie [3] and the agreement was excellent.

RESULTS

1. Measurement of absolute activity after flask combustion

Standards

Preliminary experiments with C-14 reference materials were carried out to establish the conditions necessary for quantitative recovery of carbon dioxide in Hyamine hydroxide. The results in Table 1 show that the method was quantitative and reproducible provided that at least 4 ml of Hyamine solution was used for each 100 mg total weight of sample and paper.

Plant materials

Measurement of absolute activity obtained by flask combustion of the freeze-dried cotyledons and roots are recorded in Table 2. No appreciable amount of radioactivity was accumulated in shoots.

2. Suspension counting of plant materials

Suspension counting of C-14 labelled cotyledon tissue in G/TED phosphor is shown in Fig. 2 and summarised results from suspension counting of tissue samples before and after addition of

* Panax Equipment Ltd., U.K.

** Trade name of Godfrey L. Cabot, Inc., USA

Table 1. Flask combustion of C-14 reference material

Volume of Hyamine solution (ml)	Activity of reference material (dps)	C-14 activity* recovered (dps)	Mean recovery (%)
Polymethacrylate reference material**			
2	605	391	64.6
2	475	324	68.3
3	395	384	97.3
3	535	499	93.3
4	515	520	101.0
4	485	477	98.5
5	535	530	99.0
5	645	623	96.6
6	755	775	102.5
6	515	509	98.8
n-Hexadecane reference material**			
3	665	523	78.7
4	665	667	100.2
4	625	638	102.1
4	635	643	101.2
5	565	580	102.6
5	725	727	100.2

* disintegration rate calculated from measured count rate and mean counting efficiency

** total weight of reference material with filter paper; 100 mg

Table 2. Absolute activity of plant materials by flask combustion

Tissue weight (mg)		Activity (dps)		
Fresh	Freeze-dried*	Total	per 100 mg fresh tissue	
Cotyledons				
512	95	170.9	33.4	
496	92	142.8	28.8	average;
506	94	161.0	31.6	30.8
517	96	151.0	29.2	
Roots				
157	36	73.3	46.7	
204	47	91.6	44.9	average;
139	32	68.0	48.9	46.8

* no radioactivity found in moisture condensate

internal standard, together with calculated counting efficiencies are shown in Table 3.

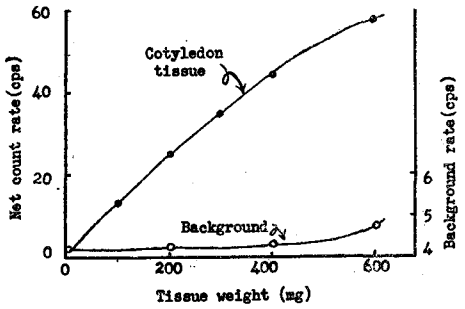

Fig. 2. Suspension counting in G/TED phosphor

Table 3. Apparent activity of plant materials by suspension counting

Tissue weight (mg)	Count rate (cps)	Internal standard (dps)	Count rate with int. stand. (cps)	Apparent* counting eff. (%)	Apparent activity of tissue (dps)	Absolute** activity (dps)	True*** counting eff. (%)
Cotyledons							
100	11.08	425	269.25	60.9	18.2	30.8	59.1
200	24.22	552	359.94	60.8	39.8	61.6	64.6
300	34.43	594	371.32	56.7	60.7	92.4	65.7
400	43.89	393	247.39	51.7	84.8	123.2	68.8
600	57.15	420	247.19	45.3	126.1	184.8	68.2
av: 19.9/100 mg							
Roots							
102	17.5	480	287.75	56.3	31.1	47.7	65.4
134	20.59	421	229.83	49.7	41.4	62.7	67.3
139	21.40	502	272.37	50.0	42.8	65.0	67.2
av: 30.8/100 mg							

* calculated from the observed count rate increment produced by a known mass of internal standard

** determined by flask combustion (refer to Table 2)

*** calculated from apparent and absolute activities

DISCUSSION

It is generally anticipated that scintillation counting of C-14 in tissue fragment suspended in liquid phosphor would suffer self-absorption loss and the calculated activity by means of efficiency value obtained, for example,by internal standardisation, would be consequently less than the true disintegration rate. The mean specific activities of cotyledons and root tissue determined by the combustion technique (Table 2) were found to be in excess of those apparent activities obtained by suspension counting (Table 3); mean ratios of $\frac{30.8}{19.9}=1.55$ for cotyledons and $\frac{46.8}{30.8}=1.53$ for roots may be derived.

The significance of this result is not the value of the ratio itself, but the fact that constancy was recorded for different plant tissues studied (the shoots were found non-radioactive). It may be inferred that a similar ratio would be obtained for other plant material. This suggests that once the ratio has been determined for a particular counting equipment, it may be used to calculate the absolute activity from the apparent activity as measured by the rapid suspension scintillation counting technique.

The constancy of the ratio confirms early work by Helf[8] who found a constant self-absorption loss although particle size of a carbon-14 labelled organic compound varied between 40 to 250 μ. Helf'osbservation and also the low value of the ratio, is contrary to expectation based on the theory of self-absorption of β-particles [1]. This theory predicts that for particles as large as those of plant tissues, the degree of self-absorption would be larger than that actually found, also that the self-absorption should be strongly dependent on variation in particle size. Since it is unlikely that all plant tissues when frozen and ground will have similar particle sizes, it appears that the results can only be interpreted on the assumption that the liquid scintillator penetrates the tissue almost completly so that it becomes part of the phosphor system; those particles which would normally be absorbed by tissue fragments are therefore able to interact with the internal phosphor.

요 약

완두유묘의 자엽 및 뿌리조직중 탄소 -14 방사능을 Liquid scintillation counter로 신속히 측정하는 새로운 방법에 관하여 실험하였다.

식물조직을 액체공기에 처리하여 분쇄한 다음 Gel phosphor에 현탁시켜, Liquid scintillation counter로 측정하였다. 이시료에 다시 내부표준물질을 가한 다음 계측효율을 구하여 Apparent absolute activity를 얻었다. 이렇게 얻은 현탁시료의 절대방사능은 자기 흡수현상때문에, 따로 Flask combustion 방법으로 구한 절대방사능 보다는 낮았으나, 항상 비례하였다. 따라서 동일한 실험조건하에서 상술한 비율을 얻으면, 완두조직중 탄소-14의 절대방사능은, 비교적 간편한 현탁측정(suspension counting) 만으로 가능하였다. 본방법은 완두 외에 다른 식물조직중 탄소-14 분석에도 쓰일 수 있다.

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