Liquid-Scintillation Counting of ^{14}C-Labelled Animal Tissues at High Efficiency

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Developments of liquid-scintillation counting as a practical method for the assay of radioisotopes in biological samples followed work by Reynolds, Harrison & Salvini (1950) and Kallmann & Furst (1950). Reviews of progress and developments in liquid-scintillation counting have been given by Davidson & Fiegelson (1957) and Bell & Hayes (1958). Guinn (1958) and Stitch (1959) have reviewed the advantages and disadvantages of liquid-scintillation counting. The advantages cited include freedom from self-absorption and window-absorption, simplicity of sample preparation, high sensitivity and reproducibility and ease of absolute calibration. Amongst the disadvantages are the high cost of the complex apparatus, the lowering of efficiency caused by phosphorescence quenching and the limitation of its use to samples soluble (or readily suspended) in the liquid scintillator. The mechanism of phosphorescence-quenching processes is discussed by Kallmann & Furst (1958).

The liquid-scintillation counting of tissues, proteins and amino acids has been accomplished in several ways. Counting of suspensions has been adopted for tissues and proteins (Hayes, Rodgers & Langham, 1956; Funt, 1956; White & Helf, 1956; Funt & Hetherington, 1957). Water-soluble substances have been blended with scintillator with 1:4-dioxan–naphthalene systems (Furst, Kallmann & Brown, 1955). The $^{14}CO_2$ from digested tissues and proteins has been trapped in methanolic solutions of the free base of the quaternary ammonium compound Hyamine 10-X. Such solutions are completely miscible with scintillator (Passmann, Radin & Cooper, 1956; Eisenberg, 1958). A solution of this free base has also been used to dissolve amino acids, and even proteins are soluble if incubated for 2 hr. at 60° (Radin, 1958; Passmann, Radin & Cooper, 1957; Steinberg, Vaughan, Anfinsen & Gorry, 1957; Vaughan, Steinberg & Logan, 1957).

The chemical procedures required for the preparation of suspensions, solutions of the free base of Hyamine 10-X and digestion of tissues are lengthy. It was for this reason that the authors sought to dissolve dried tissues and proteins directly in a solution of the chloride form of Hyamine 10-X in methanol.

The high efficiencies attained (90% for xylene-soluble sources and 60% for sources soluble in aqueous solutions), and the method of blending sources insoluble in scintillator, together with the considerable number of variables which affect the efficiency and reproducibility of both blending and counting, should be of general application and of interest to workers in this and other fields.

APPARATUS AND MATERIALS

Apparatus. The assembly [NE 8301, Nuclear Enterprises (G.B.) Ltd., Sighthill, Edinburgh 11] for scintillation counting of ^{14}C [consisting of the shielded head unit (NE 5503), stabilized E.H.T. supply (NE 5302), non-blocking linear pulse amplifier (NE 5202) and single-channel differential pulse-height selector (NE 5102)], was employed throughout the experiments described, in conjunction with the Phillips universal scaler (PW 4032) and preset count unit (PW 4052).

The pulse-height selector was operated on the integral setting at pulse-height setting of 4v. Tap water was passed continually through the cooling coils of the head unit. The activity of solutions was measured in cylindrical quartz counting vessels, 5 cm. high and 2·5 cm. diam., silvered on the outside walls and fitted with opaque plastic caps [Nuclear Enterprises (G.B.) Ltd.]. Optical contact between the window of the counting vessel and the photomultiplier-tube window (1 in. diam.) was maintained by the use of silicone oil (200/20 centistokes).

Radiochemicals. [^{14}C]Benzoic acid and [1-^{14}C]stearic acid (The Radiochemical Centre, Amersham, Bucks) were dissolved in sulphur-free xylene, and [^{14}C]glycine (The Radiochemical Centre) was dissolved in glass-distilled water.

Organic scintillator. All investigations were carried out with the NE 213 scintillator based on xylene and naphthalene. For sources soluble in xylene the scintillator was deoxygenated by N_2 through the solution, but with sources soluble in aqueous solution the scintillator was not treated in this way.

Reagents. Hyamine 10-X [2-methyl-4-(1:1:3:3-tetramethylbutyl)phenoxyethoxyethyldimethylbenzylammonium chloride monohydrate] [C. Lennig and Co. (G.B.) Ltd., London, W.C. 1] was used as a molar solution (mol. wt. 480·11) in methanol (A.R. grade). All organic solvents

employed were of A.R. grade but no special purification processes were used.

Biological samples. Liver and oviduct tissues and blood sera were obtained from chicks, some of which had received 40μc of [^{14}C]glycine/kg. live wt. by intraperitoneal injection. The tissues were sliced and the blood sera frozen before drying *in vacuo* over phosphorus pentoxide. They were then finely ground and stored under vacuum.

RESULTS

Effect of scintillator volume on counting efficiency

Xylene-soluble sources. In view of the high efficiency obtained with the NE 213 scintillator for xylene-soluble sources, no investigations of other scintillators were made. Various workers have published their findings in this aspect of the subject (Hayes, Ott, Kerr & Rodgers, 1955; Hayes, Ott & Kerr, 1956; Ott, 1958).

Scintillator volume, however, has a considerable effect on the efficiency and cost of counting samples and this was investigated in the first instance. The scintillator was deoxygenated by gassing with nitrogen before being dispensed into the counting vessel. The source was counted at increasing volumes of scintillator and the efficiency of counting determined. Fig. 1 shows the effect of scintillator volume on a source of 2μmc of [^{14}C]benzoic acid dissolved in 0·1 ml. of xylene.

The form of the curve is similar to that reported by Stitch (1959) for a [^{14}C]testosterone source dissolved in benzene. The maximum efficiency of counting was, however, some 26% higher than that reported by Stitch (1959). This efficiency has been obtained for two different samples of [^{14}C]benzoic acid, and a [1-^{14}C]stearic acid source in xylene has given a curve similar to that in Fig. 1. There was a linear relationship between scintillator volume and background count rate, the count increasing to 185 counts/min. at a volume of 15 ml. For all work described below a volume of 4 ml. of scintillator was used, corresponding with 91% counting efficiency and a background of 100 counts/min.

Sources soluble in aqueous solvents. Sources in aqueous solution have to be blended with the scintillator. We have found that it is possible to use unpurified Hyamine 10-X (chloride form) to blend aqueous samples without resource to the difficult technique of preparing the free base, and at the same time avoiding the difficulty of maintaining solutions of this base, which is liable to lose its titre (Radin, 1958). In the presence of a molar solution of commercial Hyamine 10-X in methanol, solutions of amino acids were miscible with the NE 213 scintillator. The volume of Hyamine 10-X solution used was found to be critical. If a volume of Hyamine 10-X greater than the minimum required to achieve complete miscibility was used, then additional scintillator was required to attain maximum efficiency. This aspect will be examined later. Accurate dispensing of the radioactive solutions at the low volumes was essential for reproducibility, and for this reason an Agla micrometer syringe (Burroughs Wellcome and Co., London) was employed.

The effect of increasing volumes of scintillator on efficiency of counting a [^{14}C]glycine source is pre-

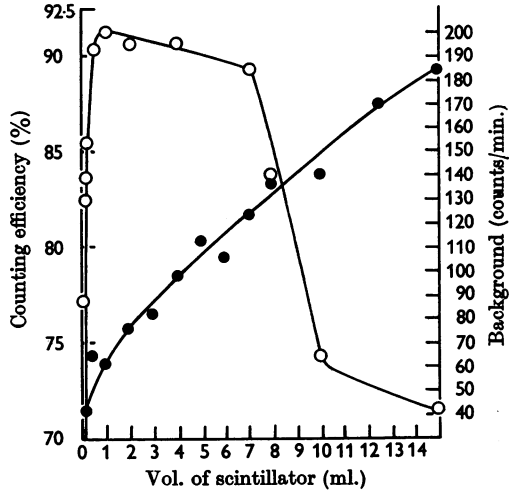

Fig. 1. Effect of increasing the volume of scintillator (deoxygenated by gassing with nitrogen) on the counting efficiency for a [^{14}C]benzoic acid source in xylene (2μmc/0·1 ml.). ○, [^{14}C]Benzoic acid source; ●, background count rate.

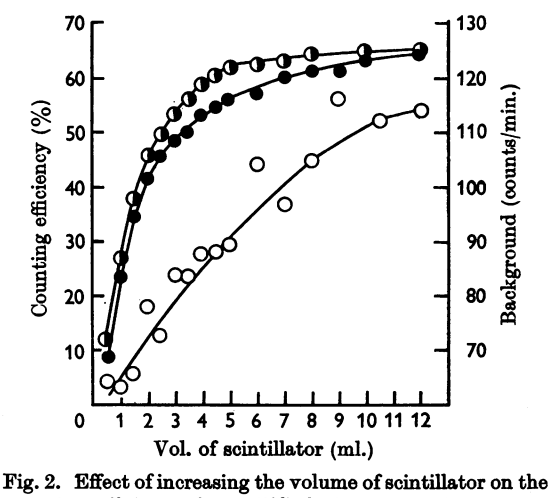

Fig. 2. Effect of increasing the volume of scintillator on the counting efficiency for a [^{14}C]glycine source in aqueous solution blended with Hyamine 10-X. ◑, 4μmc (0·1 ml.) of [^{14}C]glycine with 1·3 ml. of Hyamine 10-X; ●, 4μmc (0·1 ml.) of [^{14}C]glycine with 1·5 ml. of Hyamine 10-X; ○, background count rate.

sented in Fig. 2. The efficiency rises with increasing scintillator volume to a maximum of 65% at about 10 ml., and further additions of scintillator do not increase efficiency. This result is in contrast with the effects of scintillator volume on counting efficiency of scintillator-soluble sources. The effect of Hyamine 10-X volume on counting efficiency is demonstrated in Fig. 2. Greater efficiencies are attained at lower scintillator volumes with 1·3 ml. of Hyamine 10-X solution than with 1·5 ml. of Hyamine 10-X solution. For subsequent work with sources soluble in aqueous solutions a volume of 4 ml. of scintillator was employed, and at this volume the lower amount of Hyamine 10-X solution gave an increase in efficiency of 6%.

Phosphorescence quenching

Organic solvents. Several investigators have studied the effect on counting efficiency of organic solvents commonly used in biochemical investigations (Guinn, 1958; Stitch, 1959). We also have investigated the effect of certain organic solvents on the high counting efficiency achieved with our system. For this experiment we have used a 4μmc source of [^{14}C]benzoic acid in 0·1 ml. of xylene added to 3 ml. of scintillator. The results

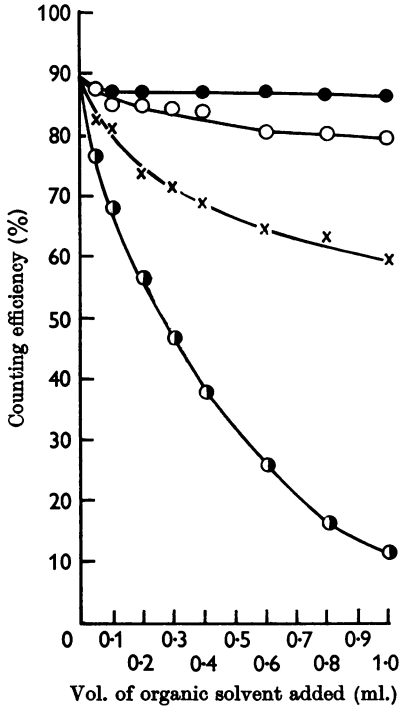

Fig. 3. Effect of the addition of organic solvents on the counting efficiency for a 4μmc source of [^{14}C]benzoic acid in 3 ml. of scintillator. ●, Diethyl ether; ○, ethanol; ×, acetone; ◑, chloroform.

(Fig. 3) can therefore be compared with those obtained by Stitch (1959), who used a similar volume of Scintipak scintillator (Nash and Thompson Ltd., London).

Diethyl ether and ethanol have only a slight quenching effect on the scintillator, but the addition of 1 ml. of acetone depresses efficiency to 60%. The effects of chloroform are similar to those reported by Stitch (1959), but less severe quenching than that recorded by Guinn (1958) and Stitch (1959) for acetone and ethanol was found in the present investigation.

Oxygen. Kallmann & Furst (1958) consider that oxygen is a more powerful quenching agent than chloroform. Pringle, Black, Funt & Sobering (1953) found that deoxygenation by gassing with nitrogen increased efficiency of counting, but this was not confirmed by Stitch (1959), who found, however, that oxygenation reduced efficiency by 10%. We have found that oxygenation of the scintillator lowers efficiency by 12% when xylene-soluble sources are counted. With sources soluble in aqueous solution blended with Hyamine 10-X the loss of efficiency on oxygenation was greatly increased, to 30%. When the scintillator was deoxygenated with nitrogen the efficiency increased by 4–5% but gradually decreased on storage (Table 1). Deoxygenation of the scintillator added to sources soluble in aqueous solution was not investigated, since continual deoxygenation of all the other components would have been necessary.

Dark adaptation to reduce light-induced phosphorescence of quartz counting vessels

The nature of the phosphorescence of quartz and glass when exposed to light has been examined by Davidson (1958), who showed that it consisted of two components, one with a half-time of 1–3 min. and the other a slow component with a half-time of 1–2 hr. Stitch (1959), counting [^{14}C]steroids, did not report this phosphorescence effect.

In our experiments the fast component of quartz phosphorescence has been found to be of impor-

Table 1. *Effect of deoxygenation on counting efficiency*

[^{14}C]Benzoic acid source (2μmc) in 4 ml. of scintillator was deoxygenated by bubbling N_2 through the solution and counted at intervals, and finally gassed again with N_2 and counted.

Time after gassing with N_2	Counting efficiency (%)
0 min.	91·2
1 day	86·7
2 days	86·8
4 days	85·9
0 min. (regassed)	90·7

tance. For example, in a source giving 4000 counts/min. there was a rapid fall of about 200 counts/min. over the first 2 min.; after this time statistical variations in the count rate tended to obscure the smaller slow component (Table 2). Thus where sources of this magnitude are present the slow component may be neglected, and for this reason a dark-adaptation time of 4 min. has been adopted for routine counting.

The magnitude of the slow component is of significance in counting sources of very low activity. When no source is present the effect of the slow component can be demonstrated. Table 3 shows the dark-adaptation of a vessel containing scintillator which had been exposed to daylight for 10 min.

Table 2. *Light-induced phosphorescence of the quartz counting vessel in the presence of a source of* ^{14}C

[^{14}C]Benzoic acid source (2 μmc) was used in 4 ml. of scintillator. Counts for the first minute after the end of relevant dark-adaptation period are shown. Before each determination the counting vial was left uncapped for 10 min. on a laboratory bench illuminated by daylight.

Time of dark adaptation (min.)	Counts/min.
0	4145
0·5	4065
1·0	4004
2·0	3960
3·0	3902
4·0	3956
5·0	3960
6·0	3956
30·0	3902
60·0	3965

Table 3. *Light-induced phosphorescence of the quartz counting vessel with no source present*

A counting vessel containing 4 ml. of scintillator was exposed to daylight for 10 min., placed in the scintillation head and counts were recorded during each minute as dark adaptation proceeded.

Time of dark adaptation (min.)	Counts/min.
1	185
2	120
3	127
4	112
5	97
6	103
7	105
8	101
9	83
10	84
Mean 11–20	80
Mean 21–30	71
Mean 31–40	67
Mean 41–50	70
Mean 420–430	50
Mean 1320–1330	45

Preparation of tissue and protein solutions for counting

After successful use of the chloride form of Hyamine 10-X for the blending of amino acid solutions with scintillator, tests were carried out to examine the value of this form for dissolving tissues.

Dry tissues. The use of N-KOH and Hyamine 10-X to dissolve tissues was investigated and the following technique was finally adopted. To 0·5 g. of finely ground tissue or protein 25 ml. of N-KOH was added and the mixture heated to boiling for 5 min. After cooling, 15 ml. of M-Hyamine 10-X in methanol was added and the solution stirred vigorously and made to a volume of 50 ml. with Hyamine 10-X solution.

Fresh tissues. The following technique was used: 2·5 g. of fresh tissue was homogenized with 15 ml. of N-KOH in a Nelco homogenizer and the mixture was transferred quantitatively to a beaker, 7·5 ml. of N-KOH being used to wash the vessel. The solution was then treated as for dry tissues. Fresh blood serum (2·5 ml.) can be dissolved directly in N-KOH (22·5 ml.) and Hyamine 10-X (25 ml.).

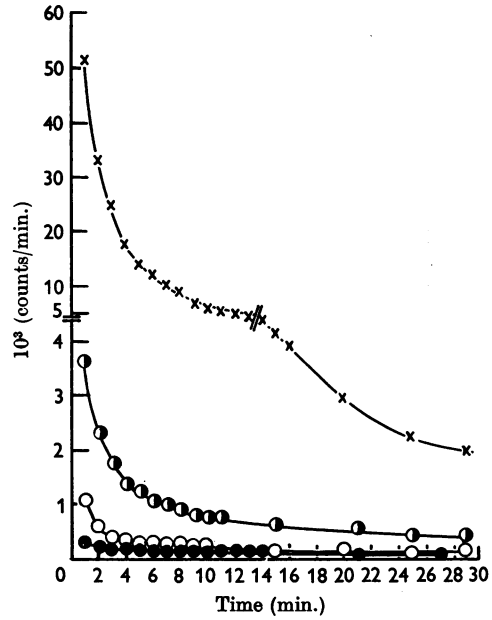

Fig. 4. Effect of acidification on the spurious counts produced by alkaline tissue solutions (0·5 ml. samples containing 0·25 ml. of N-KOH) blended with 3 ml. of Hyamine 10-X and 4 ml. of scintillator. ×, Without HCl, pH 12·00; ◑, 0·10 ml. of 2N-HCl, pH 11·55; ○, 0·20 ml. of 2N-HCl, pH 0·65; ●, 0·25 ml. of 2N-HCl, pH 0·45.

These methods produce clear and only slightly coloured solutions. With these techniques, dried blood sera from oestrogen-treated chicks, containing up to 60% of fat, have produced clear solutions for counting. Attempts to dissolve greater quantities of dry tissue than 10 mg./ml. have not been made. The method would seem to be generally applicable to a wide range of samples of animal origin.

It is not possible to blend these solutions directly with scintillator without the addition of extra Hyamine 10-X solution. Furthermore, the alkaline solution of the source produces a large spurious count rate with a very long half-time. Fig. 4 shows this high count rate for an unlabelled tissue solution and the effect of adding increasing amounts of acid to it.

Acidification of the KOH–Hyamine 10-X solution of the tissue in the counting vessel eliminated the alkali-induced scintillations. From Fig. 4 it may be seen that the highest level of addition of acid eliminated all the spurious counts so that a normal dark-adaptation curve was obtained. In routine counting therefore a volume of 2N-HCl equal to the volume of N-KOH in the sample was added. With the samples (0·1 ml.) containing 0·05 ml. of N-KOH, which we usually assay in our work, we therefore used 0·05 ml. of 2N-HCl. These acidified samples were blended with 4 ml. of scintillator, with 1·15 ml. of M-Hyamine 10-X in methanol to produce a clear solution for counting. The pH value of the acidified solution was 0·3.

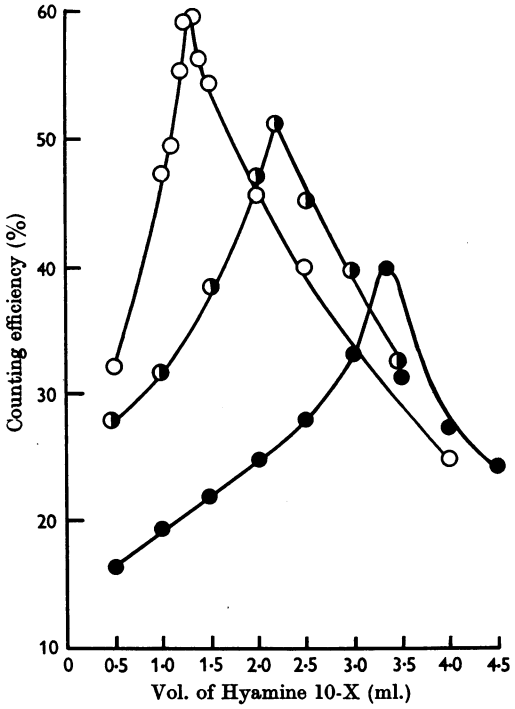

Fig. 5. Effect of Hyamine 10-X and sample volumes on counting efficiency for an amino acid. Scintillator volume was 4 ml. in each case. ○, 0·1 ml. (4μmc) of [^{14}C]glycine; ◑, 0·2 ml. (4μmc) of [^{14}C]glycine; ●, 0·4 ml. (4μmc) of [^{14}C]glycine.

Use of Hyamine 10-X *for blending aqueous samples with scintillator*

Effect of sample and Hyamine 10-X *volumes.* The presence of water in samples lowered counting efficiency. The efficiency of counting varying volumes of [^{14}C]glycine in water solution, and the effect thereon of the volume of Hyamine 10-X used for blending these solutions, was studied (Fig. 5). We have found that counting efficiency rises rapidly with addition of Hyamine 10-X until a clear solution is obtained. The addition of Hyamine 10-X solution in excess of the volume required for a clear solution causes a rapid fall in efficiency. The clarity of the

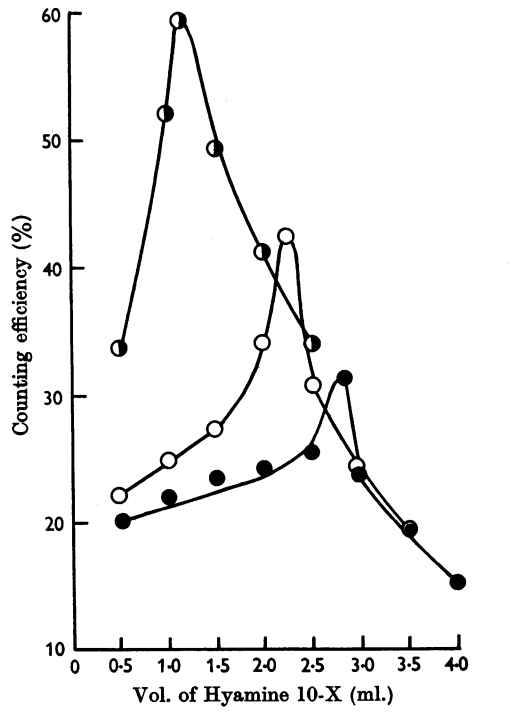

Fig. 6. Effect of Hyamine 10-X and sample volumes on counting efficiency for a tissue solution containing added [^{14}C]glycine. Scintillator volume was 4 ml. throughout. ◑, 0·1 ml. of tissue solution (containing 1·455μmc of added [^{14}C]glycine) and 0·05 ml. of 2N-HCl; total aqueous phase, 0·15 ml. ○, 0·3 ml. of tissue solution (containing 1·455μmc of added [^{14}C]glycine) and 0·15 ml. of 2N-HCl; total aqueous phase, 0·45 ml. ●, 0·5 ml. of tissue solution (containing 1·455μmc of added [^{14}C]glycine) and 0·25 ml. of 2N-HCl; total aqueous phase, 0·75 ml.

solution is checked by reading small print through the solution and the quartz window of the counting vessel. It has been found that acid hydrolysates of tissue proteins behave similarly to a solution of an amino acid for counting purposes.

A similar experiment was carried out with vacuum-dried oviduct tissue. The dry tissue was dissolved in the KOH–Hyamine 10-X solution at a concentration of 10 mg./ml. The background count rate was determined with this solution and [^{14}C]-glycine was added to measured samples at concentrations of 1·455μmc/0·1 ml., 1·455μmc/0·3 ml. and 1·455μmc/0·5 ml. The effects of sample volume and addition of Hyamine 10-X on counting efficiency with this solution is shown in Fig. 6.

Although at a sample volume of 0·1 ml. the counting efficiency for tissue is the same as for an amino acid solution, i.e. approx. 60%, at 0·3 ml. and 0·5 ml. it is 1·6% and 3% lower respectively, suggesting some degree of self-quenching associated with the increasing weight of tissue present. The lower volumes of Hyamine 10-X solution required to blend the tissue solutions, as compared with the amino acid solutions, is due to the fact that half of the tissue sample volume already consists of Hyamine 10-X solution.

Recovery of added ^{14}C *activity from tissue and protein solutions.* To check the validity of the method described for tissues, recoveries of varying amounts of [^{14}C]glycine were made from solutions of dried oviduct, liver, blood serum and egg albumin. The count rate due to the activity already present in the solutions of oviduct, liver and blood serum was included in the blank. The results are presented in Table 4.

The recoveries are stated as percentages of that achieved with a sample (0·1 ml.) of [^{14}C]glycine alone, counted under the optimum conditions for amino acid solutions. The results show that no marked quenching effects are apparent when these different materials are counted at sample volumes of 0·1 ml. and that the use of an internal standard will not generally be required, although periodic checks that the counting efficiency is being maintained are recommended.

The errors encountered in these recoveries are of an order equal to the errors normally obtained owing to statistical variation in the rate of disintegration. Thus we assume that errors due to self-quenching are insignificant. The variance in count rates of different samples was of the same order as that within samples. It will be seen from Table 4 that the highest coefficient of variation is 2·25 for the liver sample, indicating that this method is acceptable for assays of this type.

Further evidence of the absence of self-quenching when counting samples (0·1 ml.) of tissue solution is provided by the fact that mixing of active and inactive tissue solutions does not affect the efficiency of counting.

DISCUSSION

The high counting efficiency which we have obtained for xylene-soluble sources is high even for scintillation techniques. We have found that lipid extracts from animal tissues may be counted at the same efficiency as [1-^{14}C]stearic acid; details of this will be reported later.

The form of the curve obtained for the effect of scintillator volume on counting efficiency of a xylene-soluble source is similar to that reported by

Table 4. *Comparative recovery of radioactivity from varying amounts of* [^{14}C]*glycine added to different tissue and protein solutions*

Scintillator (4 ml.), 1·15 ml. of Hyamine 10-X and 0·05 ml. of 2N-HCl were used throughout with samples (0·1 ml.) of tissue solutions containing [^{14}C]glycine. The percentage efficiency obtained with 4 ml. of scintillator, 1·3 ml. of Hyamine 10-X and 0·1 ml. (4μmc) of an amino acid, i.e. 59·4%, was taken as 100% recovery.

Radioactivity added (to 0·1 ml. of sample)		Oviduct		Liver		Blood serum		Egg albumin	
μmc	Disintegrations/min.	Counts/min. (less blank)	Recovery (%)	Counts/min. (less blank)	Recovery (%)	Counts/min. (less blank)	Recovery (%)	Counts/min. (less blank)	Recovery (%)
0·3636	807	480	100·13	468	97·63	482	100·56	481	100·34
		471	98·25	473	98·67	479	99·93	490	102·22
0·7619	1691	1016	101·14	981	97·66	1027	102·24	990	98·57
		1024	101·94	1033	102·88	1018	101·35	981	97·66
1·455	3230	1915	99·81	1871	97·53	1911	99·60	1895	98·25
		1935	100·00	1876	97·78	1870	97·46	1944	101·33
1·778	3947	2279	97·21	2386	101·77	2319	98·91	2281	97·29
		2303	98·23	2401	102·41	2320	98·96	2385	101·73
2·667	5921	3510	99·80	3416	97·11	3551	100·96	3510	99·80
		3521	100·12	3461	98·40	3574	101·62	3412	97·02
Mean ± S.E.M.		—	99·76 ± 0·44 (10)	—	99·18 ± 0·71 (10)	—	100·15 ± 0·46 (10)	—	99·42 ± 0·61 (10)

Stitch (1959). This effect is important in obtaining the highest possible counting efficiency and in economizing in the use of scintillator. The background count rates which we have recorded are comparable with those obtained by Steele, Bernstein & Bjerknes (1957), Hodgson & Gordon (1958) and Stitch (1959). The relationship between background count rate and scintillator volume also emphasizes the advantages of using low volumes of scintillator. It is essential that the background count rate from each counting bottle be measured, since we have found great variability in the background count from different bottles. With 4 ml. of scintillator the background count has been found to vary between 40 and 100 counts/min. for different bottles. With samples of low activity such factors are of great importance.

With the use of Hyamine 10-X (chloride form) to blend water-soluble samples we did not encounter spurious counts as reported by Steinberg, Vaughan, Anfinsen, Gorry & Logan (1958). The effect of scintillator volume on the counting efficiency of a source soluble in aqueous solvents is very different from that for a xylene-soluble source. It is difficult to suggest an explanation for the continual increase in efficiency with scintillator addition other than the favourable influence produced by a dilution of quenching agents.

The lower background count obtained in the presence of methanolic solutions of Hyamine 10-X may be attributed to quenching by Hyamine 10-X. This can be demonstrated by using an ethanolic solution of Hyamine 10-X, when no increase in counting efficiency is obtained, even though ethanol has a smaller quenching effect than methanol.

The authors have developed this high-efficiency counting technique for small weights of tissue and protein of the order of 10–20 mg. derived from studies of the uptake of amino acids by oviduct tissue sections *in vitro*. In this case it is not possible to overcome the low counting rate of these tissues solely by increasing sample weight (a procedure that is often adopted in end-window counting). Many other metabolic experiments produce small water-soluble samples of low specific activity which could be counted more easily by this technique.

The method is reproducible provided that close attention is paid to the experimental variables that we have described. The reproducibility of the recoveries of [^{14}C]amino acids from tissue solutions shows that there is no measurable quenching due to the presence of the small weight of tissue used. For this reason internal standards need only be used occasionally to check the efficiency. In view of the lack of knowledge of quenching substances it is recommended that with any new biological sample its possible action as a quencher should be investigated.

Factors affecting reproducibility in counting xylene-soluble sources may also be mentioned. We have used a xylene-based scintillator containing naphthalene, which, according to the results and theories of Kallmann & Furst (1958), should be less susceptible to quenching by organic solvents than toluene-based scintillators. Our investigation of quenching with organic solvents confirms this. The advantage of restricting the volume of the sample counted is very obvious. Even for the most severe quencher examined, chloroform, the use of the small volume recommended will allow counting at a fairly high efficiency. Results for quenching by dissolved oxygen similarly support the theories of Kallmann & Furst (1958). These workers also state that naphthalene decreases the quenching effect of water and this may explain partly our high efficiencies with water solutions.

It may be mentioned that in order to minimize dark-adaptation problems the counting vessels were never exposed to fluorescent or ultraviolet light, nor were they placed in direct sunlight.

No reference has been found in the literature to the effect of alkalinity in producing the high spurious count rate nor to its elimination by acidification. We have found that alkalis generally produce this effect.

SUMMARY

1. The counting of ^{14}C-labelled substances, soluble and insoluble in the scintillator, at counting efficiencies of 90 and 60% respectively with a single photomultiplier liquid-scintillation-counting assembly operating at room temperature is described.

2. The effect of scintillator volume on xylene-soluble sources and sources soluble in aqueous solutions was investigated.

3. Phosphorescence quenching of the scintillator by organic solvents and by oxygen was examined.

4. The importance of phosphorescence of the quartz counting vessel in determining count rates is indicated.

5. A routine method is described for dissolving tissues and proteins by means of N-potassium hydroxide and a molar solution of Hyamine 10-X in methanol to produce solutions containing 10 mg. of dry tissue/ml. for liquid-scintillation counting.

6. The use of Hyamine 10-X for blending sources soluble in aqueous solution with scintillator is described. The effect of both the volume of Hyamine 10-X solution used for blending, and of the sample, on counting efficiency was shown with [^{14}C]glycine solutions alone and with [^{14}C]glycine added to tissue solutions.

7. The efficiency of the Hyamine 10-X blending method was demonstrated by measuring the

recoveries of added counts (as [^{14}C]glycine) from tissue and protein solutions. [^{14}C]Glycine dissolved in these solutions was counted at the same efficiency as in water solution.

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The Zinc Content of Erythrocytes and Leucocytes of Blood from Normal and Leukaemic Subjects

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The biological importance of zinc is now well established, and the significance of zinc in the plasma and cellular components of blood has been studied by many investigators. Human leucocytes contain as much as 3% of the whole-blood zinc (Vallee & Gibson, 1948), and the individual leucocyte is relatively rich in this metal and probably contains more zinc than does any other cell in the body (Vikbladh, 1951). For example, normal human leucocytes contain about twenty-five times as much zinc per cell as do erythrocytes (Vallee & Gibson, 1948; Wolff, 1956). A protein which contains 0·3% of zinc, and which accounts for about 80% of the zinc of the leucocytes, has been extracted from normal human leucocytes (Hoch & Vallee, 1952; Vallee, Hoch & Hughes, 1954). Although it is suggested that this zinc-protein is probably an enzyme, the nature of its enzymic activity is unknown.

In investigations on the transport of zinc in the blood and other aspects of zinc 'metabolism', we have become interested in the zinc content of