SCINTILLATION COUNTING OF ^{32}P WITHOUT ADDED SCINTILLATOR IN AQUEOUS SOLUTIONS AND ORGANIC SOLVENTS, AND ON DRY CHROMATOGRAPHIC MEDIA

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ABSTRACT

SCINTILLATION COUNTING OF ^{32}P WITHOUT ADDED SCINTILLATOR IN AQUEOUS SOLUTIONS AND ORGANIC SOLVENTS, AND ON DRY CHROMATOGRAPHIC MEDIA

By

Randolph Thompson Haviland

Cerenkov radiation produced by ^{32}P makes possible a convenient and efficient method for determination of ^{32}P in virtually any sample utilizing conventional scintillation counters. Organic solvents provide high absolute efficiencies -- the highest efficiency measured, approximately 80%, was obtained with toluene. Approximately 50% absolute efficiency is obtained in aqueous solutions with very little variation when strong acids, bases, or salts are present. Paper and silica gel give similar efficiencies with no liquid present because Cerenkov radiation occurs in polyethylene and in glass. Samples are completely unaltered by the counting. Counting conditions are constant for all samples. The efficiency varies with volume, solution composition, and vial material. Efficiencies relative to water for a wide variety of aqueous solutions and organic solvents have been determined using a rapid, highly accurate method.

SCINTILLATION COUNTING OF ^{32}P WITHOUT USING SCINTILLATORS

IN WHOLE SCINTILLATION COUNTING SOLUTIONS,

IN OTHER CASES POSSIBLE WITH

By

Lincoln Thompson Levi-Strauss

A THESIS

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INTRODUCTION

Detection of radioactive nuclides giving rise to Cerenkov radiation in aqueous solution employing conventional liquid scintillation spectrometers has been demonstrated in recent years (1, 2). The accuracy of the method when applied to ^{3}H has been shown to be essentially equivalent to that obtained with normal scintillation counting (3), and the use of channels ratio and external standardization techniques for color quench correction have been noted (3, 4). The studies published to date have examined only a limited number of aqueous (2, 3, 4) and organic solvent (5) systems. An examination of the physical parameters involved in Cerenkov counting of a number of isotopes, including ^{32}P, has been published (9). Though participation of the vial in Cerenkov counting of γ emitters is demonstrated, the significant contribution made by the vial material in counting energetic β emitters such as ^{32}P, is not noted (9). Directional response of Cerenkov radiation limits efficiency in the coincidence mode of conventional scintillation counters (9). However, an entirely new and complicated instrument would be required to overcome this problem and provide maximum Cerenkov efficiency (9), and the high efficiency obtained for ^{32}P with conventional instruments suggests that such considerations are of relatively limited importance. Following the observation in this laboratory that ^{32}P-labeled phospholipids in chloroform as well as ^{32}P in water can be counted utilizing Cerenkov radiation, a thorough examination of the method has been undertaken.

MATERIALS

Carrier-free ^{32}P as $H_3{}^{32}PO_4$ in 0.02 N HCl and a ^{32}P absolute standard, NAS-017, as 0.01 N $H_3{}^{32}PO_4$, were obtained from New England Nuclear Corporation. Salmon-sperm DNA ("highly polymerized" from Sigma) and purified yeast RNA were gifts from Dr. J. L. Fairley. Whatman No. 1 paper and E. Merck glass-backed silica gel G plates (Brinkman Instruments, Inc.) were employed for the dry counting experiments. All other chemicals were reagent grade, and distilled water was used throughout.

Eighteen ml capacity polyethylene vials (Beckman Instruments, Inc.) were generally used, although 25-ml polyethylene vials and 20-ml glass vials (Packard Instrument Co.) were also employed as indicated. Unless otherwise specified, counting was performed using a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3310. All samples were maintained at 4°C in the refrigerated counting chamber.

EXPERIMENTAL PROCEDURE AND RESULTS

Instrument conditions: optimum volume, absolute efficiency

Peak height spectra of ^{32}P in water at various gain settings (Fig. 1) reveal that significant portions of the spectrum lie below 50 and above 1000 (arbitrary discriminator divisions), the finite limits normally employed. The background at an "infinite" discriminator setting (0 - ∞) is 30 C P M at maximum (100) gain (Table I). Not only do these conditions provide maximum efficiency, but they render the count rate essentially independent of gain (Fig. 2). At infinite discriminator and maximum gain settings, the only variable with changes in the counting "system" (vial and contents) is the efficiency. Finite discriminator settings require determination of gain optima which vary with vial material, solution composition (Fig. 2), and volume, though background is reduced to 12 C P M (Table I).

Efficiency varies with sample volume both because of the counting chamber and vial geometry and because the "system" absorbs a fraction of the β-radiation. Optimum volumes are 10 ml in 10-ml polyethylene vials, 15 ml in 25-ml polyethylene vials, and 10 ml in 20-ml glass vials. The dependence of efficiency on volume (Fig. 3) restricts the volume ranges within 1% efficiency of the optimum volume to 8-13 ml in 10-ml polyethylene vials, 14-19 ml in 25-ml polyethylene vials, and 7-11 ml in 20-ml glass vials. These values may vary by ±2 ml depending on the counting chamber geometry of any particular instrument. Table I shows the absolute efficiency in water at finite and infinite settings and indicates the range observed with six instruments other than the one used.

Fig. 1 Cerenkov Peak Height Spectrum: $^{32}P_i$ in Water in Polyethylene Vials.

10 µl of stock $^{32}P_i$ in 10^{-6} M $Fe_2(TO_4)_3$, pH 7.5, in 10 ml water was counted in an 18-ml polyethylene vial. Spectral sections of 50 arbitrary discriminator divisions were counted at the gain settings indicated.

The third channel was held fixed at infinite discriminator (0 - ∞) and maximum gain (100%) settings, and counting events were terminated when this channel registered 10^5 counts. Counts in the spectral sections are expressed as percentages of the sum of counts over all sections at the indicated gain.

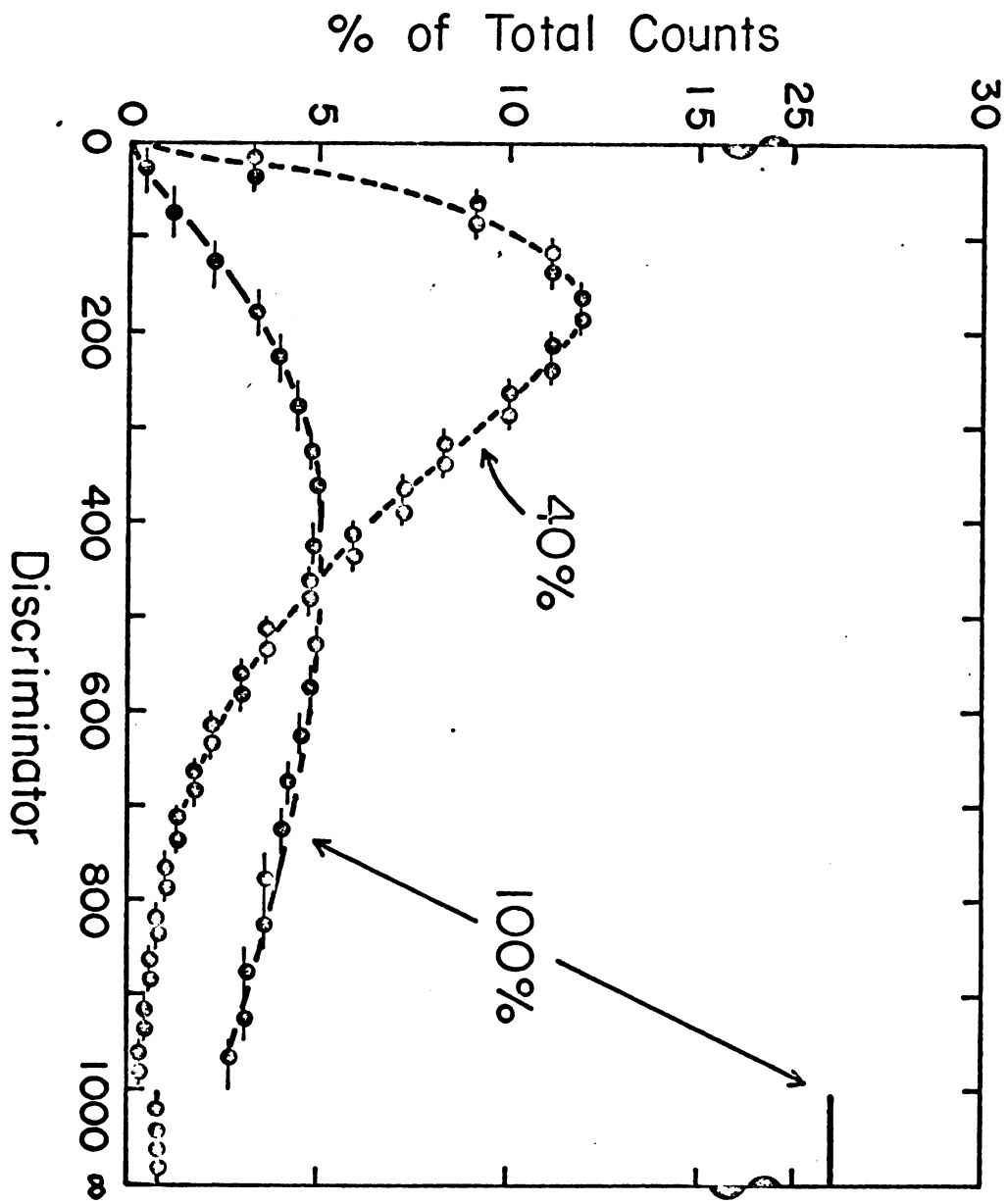
% of Total Counts
0
5
10
15
25
30
0
200
400
600
800
1000
Discriminator
40%
100%

Figure 1

Table 1 Absolute Efficiency: Cerenkov Counting of ^{32}P in Water in Polyethylene Vials

98 ± 0.8 µl aliquots of the absolute standard were added to 9.9 ml of water in 15-ml polyethylene vials. Pipettes were calibrated by filling with water at 24°C, dispensing, and weighing the aliquot. The Documenta Geigy Isotope Decay Tables (7) were used for decay correction. Samples were equilibrated to temperature for one hour before counting in refrigerated instruments. Thirty samples were used for all determinations. An average of 3.5×10^4 C M was observed. Ten-minute counts on fifty vials containing 10 ml water were used for the background determination. The absolute errors include the ±4% uncertainty in the absolute standard, the uncertainty in the pipette calibration, and the sample deviation of the counts from 30 samples (a measure of both pipetting and counting errors) propagated according to the theorem of error propagation (5). The uncertainty in the decay correction is negligible with respect to the other errors.

TABLE 1

Absolute Efficiency: Cerenkov Counting of ^{32}P in Water in Polyethylene Vials

Instrument Conditions		Absolute Efficiency	Bkgd (CPM)	E^2/B
Gain	Discriminator			
42%*	50-1000	46.5 ± 3.6%	12.7 ± 2	278
100%*	0-∞	48.1 ± 3.9%	77.9 ± 3	113
Range obtained with 6 instruments at maximum gain and infinite discriminator settings.		45.9 ± 3.8 - 51.6 ± 4.2%		

*Packard 3310 employed.

Fig. 1. Gain Optima for Various Substances in Polyethylene Vials.

I: Infinite Discriminator Setting (0 - ∞).

F: Finite Discriminator Setting (50-1000).

10 µl of stock ^{32}P in 10^{-6} M Br_2HPO_4, pH 7.5 (water), or isobutanol saturated with Na_2HPO_4 (solvents) was mixed with 10 ml of the solutions indicated and counted in 10-ml polyethylene vials. One channel of the instrument was maintained at 0 - ∞ discriminator and 1000 gain settings, and the gain of the other two channels was varied at the indicated discriminator settings. Counting events were terminated when the fixed channel registered 10^5 counts. Counts as a function of gain are expressed as percentages of the maximum count obtained at optimum gain for the particular discriminator setting. Every third point is plotted.

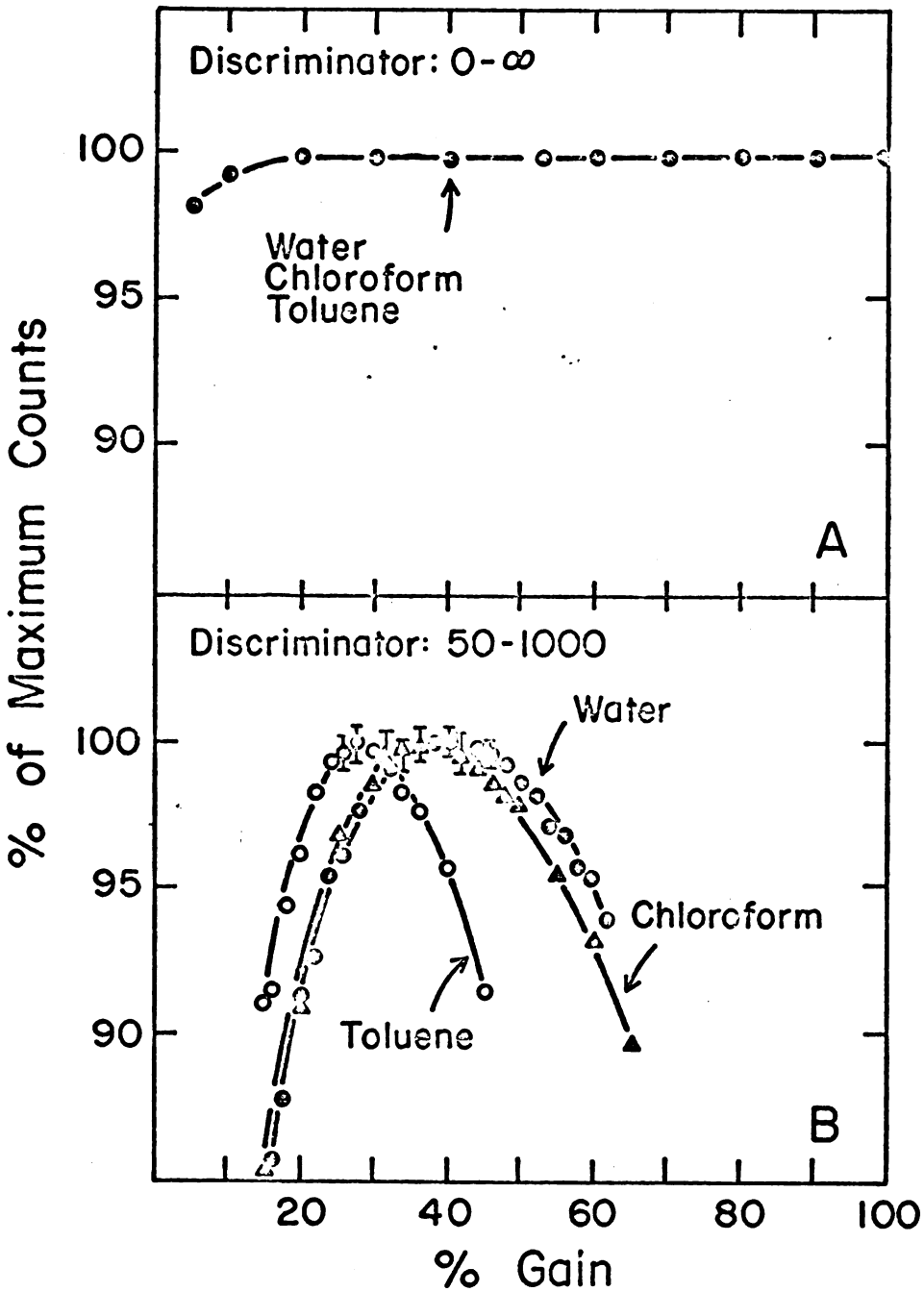
Discriminator: 0-∞
100
95
90
Water
Chloroform
Toluene
A
Discriminator: 50-1000
100
95
90
Water
Chloroform
Toluene
B
% of Maximum Counts
20
40
60
80
100
% Gain

Figure 2

Fig. 3 Optimum Counting Volume

10 µl of stock $^{3}H_2O$ in 10^{-2} M K_2HPO_4, pH 7.5, was mixed with 1 ml of water in vials of the types indicated. Volume was increased in 1.0 ml increments to the maximum capacity of the vial. One minute counts were taken at 0 - ∞ discriminator and 100% gain settings (the same results obtain at finite discriminator and optimum gain settings). 5×10^4 cpm were obtained at minimum efficiency. All materials were maintained at instrument temperature in the refrigerated counting chamber. Counts as a function of volume are expressed as percentages of the maximum count obtained at optimum volume for the particular vial. Uncertainty due to counting error is ±0.5%.

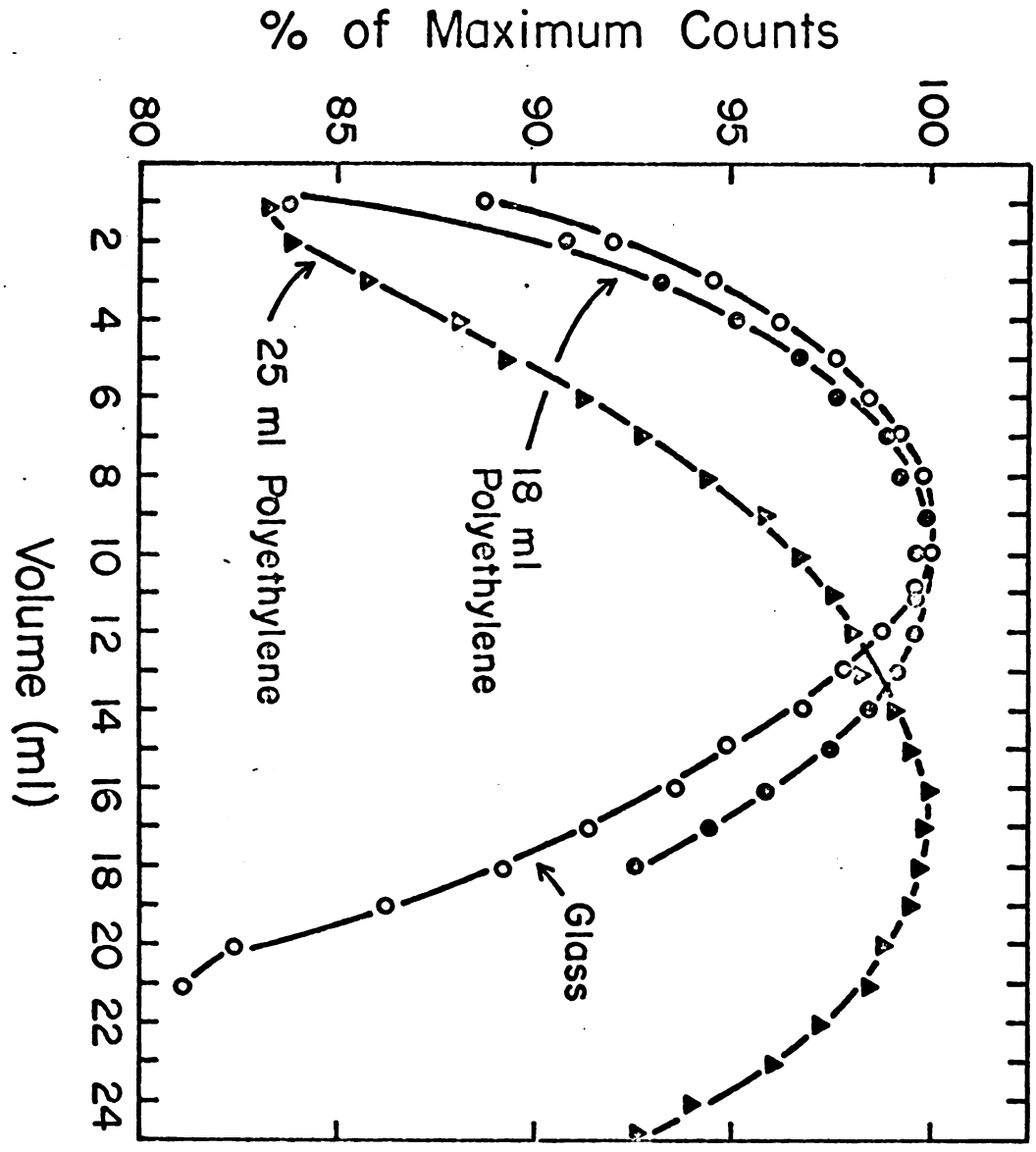
% of Maximum Counts
80
85
90
95
100
Volume (ml)
2 4 6 8 10 12 14 16 18 20 22 24
25 ml Polyethylene
18 ml Polyethylene
Glass

Figure 3

Relative Efficiencies Experiments with vials which contained dried ^{32}P in false bottoms demonstrated that Cerenkov radiation occurs in polyethylene and glass, which suggests that continuous flow counting can be performed with a coil of tubing in the counting chamber. Further, counting of samples smaller than 1 ml is possible if the small volume can be positioned reproducibly (Fig. 4). Since ±4% errors in the volume of a 10 µl aliquot change the relative counting efficiency by ± 0.4%, uncertainty due to pipetting error is considerably reduced by counting a 10-µl aliquot, adding 10 ml of a solution of known or unknown efficiency and recounting instead of pipetting 10-µl aliquots into solutions and counting. Tables 2 and 3 give relative efficiencies determined by this method.

Since low solubility of $^{32}P_i$ in solvents might have resulted in precipitation, the relative efficiencies for several solvents were checked using 1 cm^2 pieces of IE-8 paper saturated with $^{32}P_i$, rinsed with water, and dried. IE-8 paper does not release orthophosphate to 10 ml of water, so a factor relating dry counts to counts in water could be developed. The results agree within experimental error with those reported in Table 3. Different lots of chloroform and methanol gave different relative efficiencies because of varying levels of colored impurities (σ of 3 different lots of methanol was ±3%). The relative efficiencies reported for these solvents are the highest ones obtained.

Fig. 4 Volume Dependence of ^{32}P Cerenkov Counting.

10 µl of stock ^{32}P in water was pipetted into an 18-ml polyethylene vial and a sequence of appropriate aliquots of water was added. One-minute counts at 0 - ∞ discriminator and 100% gain settings were taken. Results are expressed as percentages of the CPM at 10-ml volume. Vertical bars indicate uncertainty due to counting error and position asymmetry, which was assessed by counting the vial in at least 5 positions, averaging and computing the sample deviation. ≥5 x 10^4 CPM were observed at minimum efficiency.

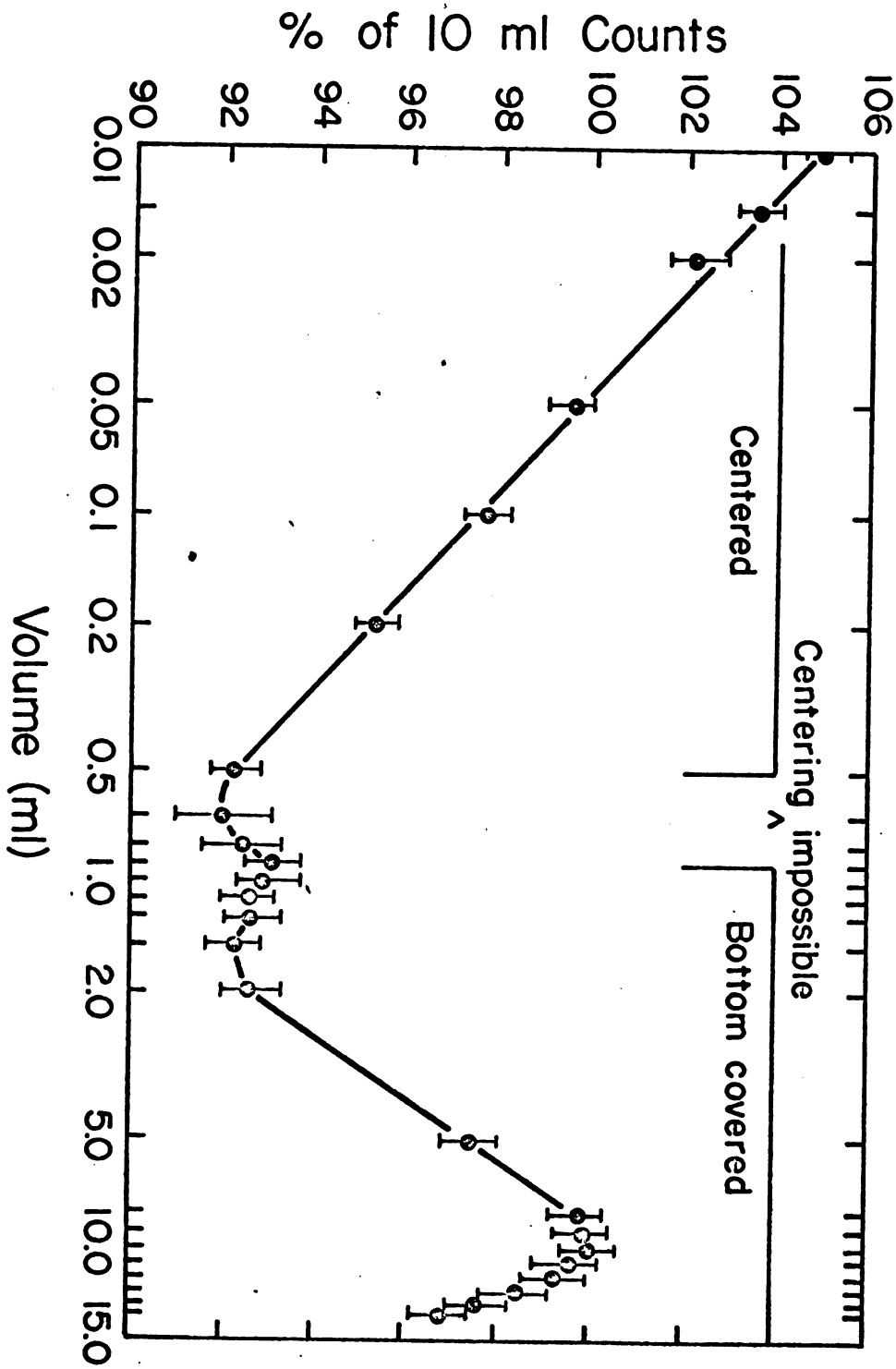
% of 10 ml Counts
90
92
94
96
98
100
102
104
106
0.01
0.02
0.05
0.1
0.2
0.5
1.0
2.0
5.0
10.0
15.0
Volume (ml)
Centered
Centering impossible
Bottom covered

Figure 4

Legend for Table 2

Depressions sufficiently large to contain 10 µl were made in 18-ml polyethylene vials by touching with a heated glass rod. Since it was difficult to center the depressions, marks were made on the outsides of the vials, and the vials were always counted in the same position. 10^{-2} M Na_2HPO_4, pH 7.5, was used as carrier for the acids, bases, and $MgCl_2$, and 10^{-6} M Na_2HPO_4, pH 7.5, was used for the other aqueous systems. 10 µl of ^{32}P in carrier was added to the empty vial, counted 5 times for 1 minute, 10 ml of the appropriate solution was added, and 5 more 1-minute counts were obtained. Factors were developed relating the counts in 10 µl to the counts in 10 ml of water for each vial and each aliquot composition, and relative efficiencies were computed by:

$$\text{R.E.} = \frac{\text{counts in 10-ml sample}}{\text{counts in 10-µl} \times \text{factor}} \times 100 =$$

$$\frac{\text{counts observed in 10-ml sample}}{\text{counts which would have been observed had 10-ml water been present}} \times 100$$

The 10-µl aliquots contained 10^5 cpm. The absolute errors are either the propagated counting error and vial calibration uncertainty, or the sample deviation of a minimum of three independent determinations, whichever was larger.

TABLE 2

Aqueous Solution Efficiencies Relative to Water

Material	Relative Efficiency (%) Discriminator 0 - ∞ 100% Gain
H_2O, glass vials	74.0 ± 1.7%
1.0 M ZnCl, 2 M NH_4OH, 1.0 M NaOH	97.7 ± 0.5, 99.1 ± 0.5, 99.4 ± 0.6%, respectively
1.0 M NaOAc, 1.0 M H_2SO_4, 1.0 M HCl	99.1 ± 0.6, 99.5 ± 0.6, 101.2 ± 0.6%
0.1 M Tris, pH 7.4	99.5 ± 0.7
0.025 M HEPES, pH 8.0	99.3 ± 0.7
0.1 M Na_2HPO_4, pH 7.2	99.5 ± 0.6
1.0 mM $MgCl_2$, 0.5 M NH_4OAc, 5.0 M NH_4OAc	98.8 ± 0.6, 99.9 ± 0.6, 106.7 ± 0.6
1.0 M Et_3NHHCO_3, pH 7.5	100.9 ± 0.5
1.0 mM [illegible]	97.3 ± 0.5
0.5 mg/ml; (Na)[illegible]	95.0 ± 0.6, 93.1 ± 0.6
1.0 M CsCl, 1.0 M Sucrose	100.6 ± 0.5, 103.0 ± 0.6

a Background ≈ 300 cpm, due to the presence of ^{40}K, a long-lived natural β-emitting isotope (E_{max} = 1.33 MeV); all other backgrounds ≤30 cpm.

TABLE 3

Solvent Efficiencies Relative to Water

Material	Relative Efficiency (%) Discriminator 0 - ∞ 100% Gain
Methanol	107.5 ± 0.6
Ethanol (95%)	115.7 ± 0.6
Acetone	119.4 ± 0.5
Petroleum Ether (b.p. 40-55°C)	121.1 ± 0.6
Hexane	128.2 ± 0.6
Benzene	159.2 ± 1.0
Toluene	166.2 ± 2.0
Chloroform	113.8 ± 0.7
$CHCl_3$:CH_3OH 1:1 (v/v)	106.0 ± 1.2
$CHCl_3$:CH_3OH 2:1 (v/v)	110.8 ± 0.5
$CHCl_3$:CH_3OH:H_2O 20:9:1 (v/v)	111.8 ± 0.7
$CHCl_3$:CH_3OH 2:1 (v/v) + 0.3% HCl, neutralized with NaOH	112.4 ± 0.6
$CHCl_3$:HOAc 3:1 (v/v)	113.1 ± 0.6
$CHCl_3$:CH_3OH 4:1 (v/v) + 10 ml/l NH_4OH	109.9 ± 0.7

TABLE 3 (continued)

Solvent Efficiencies Relative to Water

Material	Relative Efficiency (%) Discriminator 0 → 100% Gain
0.5 M NH_4OAc in $CHCl_3$:CH_3OH:H_2O 70:9:1 (v/v)	110.6 ± 0.6
1.0 M NH_4OAc in $CHCl_3$:CH_3OH:H_2O 70:9:1 (v/v)	110.0 ± 1.1

Legend: Experiments were performed as indicated in the legend to Table 2, except that the 10-µl aliquots consisted of ^{32}P in isobutanol saturated with Na_2HPO_4.

Dry Counting[1] Since Čerenkov radiation occurs in the vial walls, ^{32}P on paper or silica gel can be counted with no liquid present. Self-absorption reduces efficiency with increasing area or mass, as shown in Figure 5. Cutting the paper rectangles into pieces ~2 x 5 mm reduces the error due to position variation for one counting event from ± 10% to ± 5% (Table 4). Addition of liquid to the vials generally increases efficiency, but does not reduce th position error except when the liquid elutes a significant fraction of the radioactivity (Table 4). Glass vials gave approximately 60% efficiency relative to polyethylene vials for chopped paper (Table 4). Spraying the papers with color-forming reagents reduces efficiency, probably because of increased selfabsorption.

Indirect Efficiency Determinations Quenching of Čerenkov radiation by colored solutions can be assessed by the usual channels ratio (4) and external standardization (3) techniques. Our results (Figure 6) confirm those of White and Ellis on the utility of the external standard for color quench correction, provided measures are taken to avoid the observed ± 2% variation in external standard counts due to variation in polyethylene vial characteristics. Glass vials exhibit variation of less than or equal to ± 1%. Sample volume must be held constant. If counts are too low for accurate channels ratio

[1] While this work was in progress, Hülzen and Prenzel (6) reported counting of ^{32}P on membrane filters without liquid. Our independent experiments confirm and extend their results.

Fig. 5 Cerenkov Efficiency Relative to Water for Dry Paper and Silica Gel as a Function of Area and Mass.

10 µl aliquots of stock ^{32}P in 10^{-2} M Na_2HPO_4, pH 7.5, were pipetted into 10 ml of water (15 samples each for paper and silica gel) or on to appropriate rectangles of Whatman No. 1 paper or silica gel G plates (E. Merck, glass-backed). The rectangles were then flooded with methanol, dried, and counted in 15-ml polyethylene vials at 0 - ∞ discriminator and 100% gain settings. This procedure gave a non-uniform distribution of radioactivity similar to that obtained after partition chromatography. "Chopped" paper was cut into pieces ~ 2 x 5 mm. Unchopped rectangles of paper were positioned either flat on the bottom of the vials (1 and 2 cm^2) or around the circumference, but not completely covering the entire inside wall (5-15 cm^2), and were not crumpled. Each point represents the average of 5 samples divided by the average of the 15 standards for the material. Vertical bars represent uncertainty due to sample asymmetry and pipetting error, determined by propagation of the sample deviations for the samples and the standards corresponding to each point. A minimum of 3×10^4 CPM were detected.

P: Paper and silica gel

I: Silica gel only

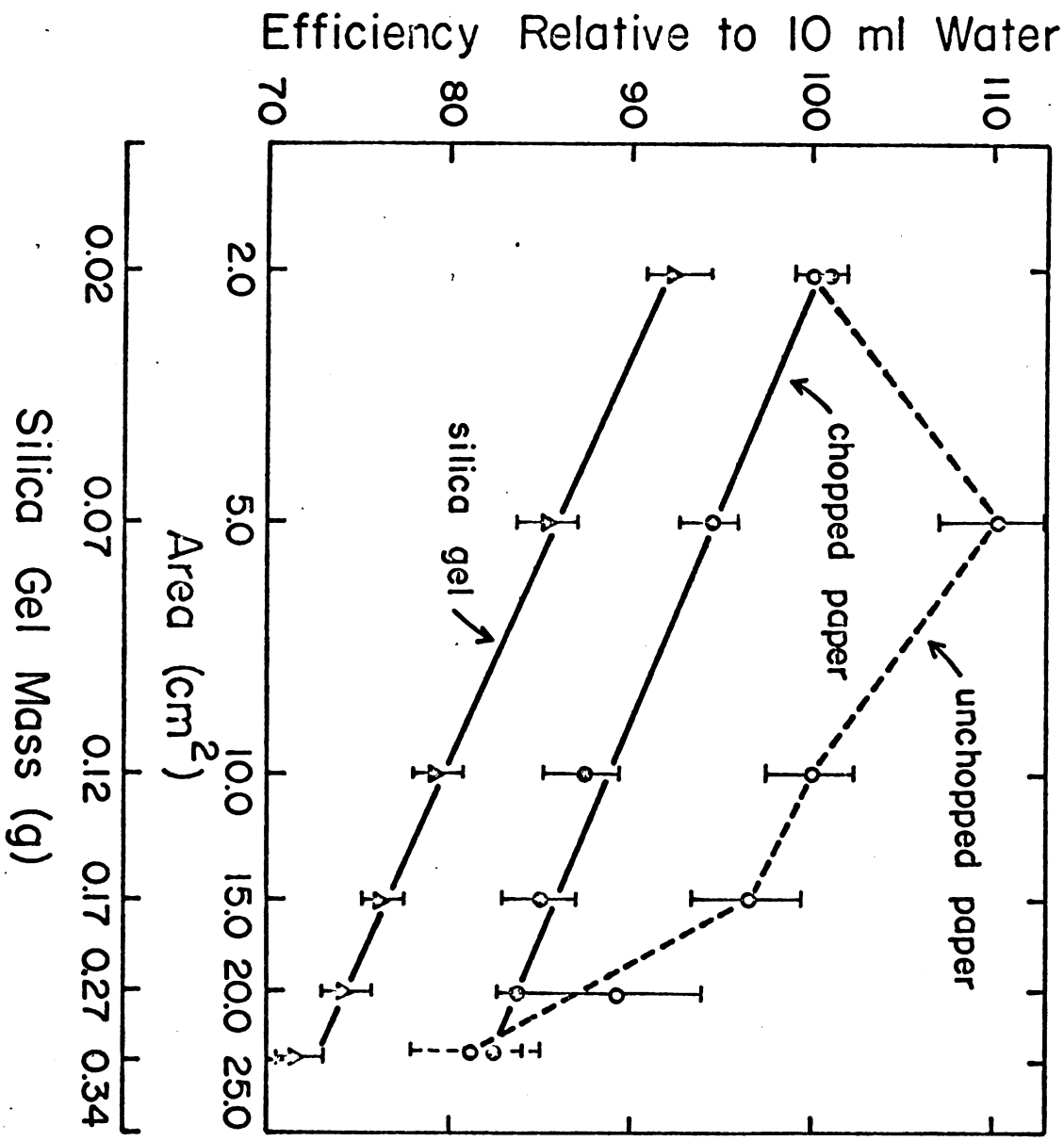
Efficiency Relative to 10 ml Water
70
80
90
100
110
2.0
5.0
10.0
15.0
20.0
25.0
Area (cm²)
0.02
0.07
0.12
0.17
0.27
0.34
Silica Gel Mass (g)
silica gel
chopped paper
unchopped paper

Figure 5

Legend for Table 4

Samples prepared as in Figure 5 were counted a minimum of ten times for 1 minute with agitation of the sample between counting events, and sample deviations computed. Aliquots were then added, or samples were placed in glass vials and the procedure repeated after a 5-minute equilibration period. A minimum of 2 x 10^4 cpm was detected at minimum efficiency with 0 - ∞ discriminator and 1000 gain settings. All materials were maintained at instrument temperature.

[1] Sample deviations are the worst cases observed from 10-1 minute counts with intermediate agitation on 3 different samples. If one count on one sample is performed, the true (average) value lies within ± 2σ of the value obtained.

[2] Average of 5,074 ± 5%.

[3] Propagated position error for all relative efficiencies: min ± 2%; max ± 6%.

[4] Cut into pieces ~2 x 5 mm.

TABLE 4

Counting Errors for Paper and Silica Gel With and Without Liquid

PAPER

Area (cm^2)	Counting error in σ (σ^2)				
	In Volvetriylene				In glass
	Unchowed	Chowed[4]	10 ml toluene	10 ml H O	chowed
2	±7%	±1.6%	±0.9%	±0.7%	±2.3%
5	2	1.1	0.9	1.2	2.2
10	3	1.4	1.2	0.3	1.3
15	6	1.6	1.2	0.4	1.4
20	7	2.5	2.0	0.5	1.0
25	6	2.3	2.4	0.6	3.3

SILICA GEL

Area (cm^2)	Mean (σ)	Counting Error (σ) in Volvetriylene (σ^2)		
		Dry	10 ml Toluene	10 ml H O
2	0.02	±1.6%	±0.8%	±0.5%
5	0.07	0.5	0.6	0.3
10	0.12	0.8	0.5	0.9
15	0.17	0.8	0.6	1.0
20	0.27	1.7	0.8	1.4
25	0.31	1.0	1.4	2.5

TABLE 4B

Efficiencies for Paper and Silica Gel with Liquids Relative to Dry Efficiency

PAPER

Area (cm^2)	Relative Efficiency to Spread in Polyethylene ( )		
	In Glass	In Polyethylene	
	dry^3	10 ml toluene	10 ml H_2O
2	50.1	138.7	99.0
5	52.7	156.0	104.5
10	59.3	161.4	111.9
15	60.7	161.8	114.3
20	60.9	165.4	114.5
25	60.3	173.5	114.0

SILICA GEL

Area (cm^2)	Mass (g)	Relative Efficiency to Polyethylene ( )	
		10 ml toluene	10 ml H_2O
2	0.03	141.6	102.4
5	0.07	160.4	109.3
10	0.13	168.6	117.3
15	0.17	176.3	119.9
20	0.27	184.0	121.7
25	0.34	193.9	129.6

Fig. 6 Color Quenching of Cerenkov Radiation

A series of 10 ml polyethylene vials containing 10 ml water was counted five times for 1 minute with the external standard at 0 - ∞ discriminator and 100% gain settings. The colored solutions were then added, and the vials recounted, and the ratio:

$$\frac{\text{counts with 10 ml quencher}}{\text{counts with 10 ml water}}$$

was computed with ± 1% uncertainty due to the observed counting error, which may have been caused by variations in vial wall thickness. Relative efficiencies to water were determined on the same samples used with the external standard by the method given in Table 2 with uncertainty due to counting error of ± 0.5%. Concentration ranges were as follows: Methylene blue, 10^{-4} - 10^{-6} M; $KMnO_4$, 10^{-3} - 10^{-5} M; $K_2Cr_2O_7$, 10^{-1} - 10^{-3} M. A minimum of 10^4 cpm was observed.

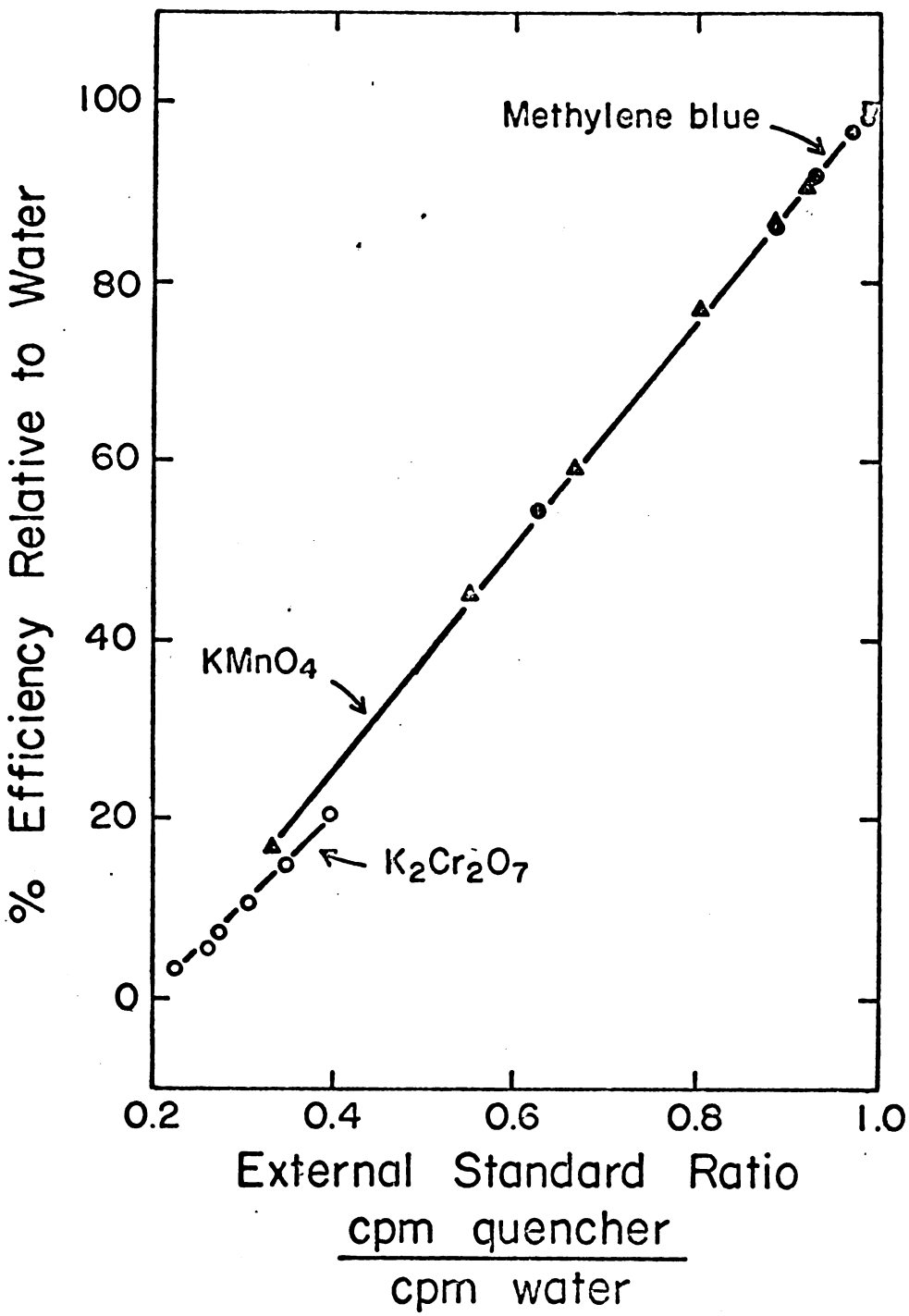
100
80
60
40
20
0
% Efficiency Relative to Water
Methylene blue
KMnO4
K2Cr2O7
0.2
0.4
0.6
0.8
1.0
External Standard Ratio
cpm quencher
cpm water

Figure 6

determination and an external standard is not available, a plot can be constructed relating optical density to counting efficiency in colored solutions. Variation in peak height spectra with vial raises the error in a determination of quenching by a channels ratio applied to the external standard counts to 4%.

Determination of relative efficiencies in colorless solutions using the external standard has not been possible. Although changes in external standard counts occur with changes in system composition, the variation is partially dependent on factors independent of the Čerenkov efficiency, probably variation in collision cross-section for Compton scattering and pair production. Even when the variation in solution composition is defined (e.g., a gradient of NH_4OAc in water), the variation in external standard counts due to variation in glass vials makes it impossible to determine relative efficiency changes smaller than 4%. Peak height spectra of either the external standard, ^{32}P in solution, or dry, show too little variation with efficiency changes for a channels ratio to be useful.

DISCUSSION

Implications and limitations of the results. The relatively broad gain optima observed in aqueous solutions (Fig. 2) and direct data not presented indicate that colorless aqueous solutions can be counted at the gain optimum for water and finite window with relative efficiencies essentially identical to those obtained at infinite window. The small changes in relative efficiency observed with wide variation in aqueous solution composition (93-106%, Table 2) indicate that for routine work not requiring greater than ±5% accuracy, the change in efficiency with changes in solute concentration can be neglected. Changes in relative efficiency in different mixtures of chloroform and methanol are also small enough to be neglected (Table 3).

The exponential relation between count rate and volume from 0.01 to 0.5 ml shown in Figure 3 is consistent with absorption of a fraction of the β-radiation by the water, while geometrical factors (centering and height of the column of water relative to the photomultiplier tubes) appear to be the major determinants above 0.5 ml. The relative efficiency of 1 M CsCl (100.6%, Table 2) indicates that self-absorption can have considerable effect on the observed efficiency, since the high refractive index of this solution ($n \cong 1.35$) should have resulted in increased Čerenkov efficiency. The vial material also affects the efficiency, as is apparent from the data in Table 2. The observed CPM can thus be represented by:

$$cpm = f_i(n_1 e_s + n_2 e_v)\ dpm$$

where f_i is the detection efficiency of the instrument, e_s and e_v are the Čerenkov efficiencies of the solution

and vial, and n_1 and n_2 are geometrical factors which vary with volume and vial construction. The resulting relative efficiency expression is:

$$RE = \frac{n_1 P_s + n_2 \epsilon_v}{n_1 P_s Q + n_2 \epsilon_v}$$

Therefore, the relative efficiencies in Tables 2 and 3 vary with volume and vial material but are independent of the absolute efficiency provided by any individual instrument. They apply to a range of 1-10 ml in 18-ml polyethylene vials and are no more than 1% below the relative efficiency at 15 ml. In 20-ml glass vials, relative efficiencies in 10 ml volumes are from 3-6% higher than those reported, and increasing volume in the range 1-15 ml causes increases in relative efficiency of 2-10%.

Precautions Since a significant fraction of the total counts with 10-ml sample volumes is due to the vial, vial characteristics must be held constant for the method to be reproducible. Polyethylene vials which have been used for scintillation counting must not be used for Cerenkov counting unless they have been extensively soaked in solvents (e.g., chloroform) to remove scintillator contained in the vial walls. Variable increases in nonsarent Cerenkov efficiency of up to 15% have been observed with polyethylene vials soaked in toluene or dioxane PPO-POPOP scintillator, and prolonged soaking in hot water and detergent does not cause a significant decrease in the level of contamination. Though there was no detectable change in efficiency or peak height spectrum for ^{14}C counted in toluene or dioxane scintillator in vials containing absorbed scintillator, the external standard counts increased by as much as 46%, and the peak height spectrum with the external standard was sufficiently distorted to produce a 70% change in a preset channels ratio.

Since most automatic external standardization systems depend on a channels ratio with the external standard, absorbed scintillator must be removed from the walls of polyethylene vials used for normal scintillation counting.

The permeability of polyethylene to organic solvents poses a more general problem since solutes may be deposited in the vial walls if solutions remain in the vials for more than a few hours. No significant changes in Cerenkov efficiency have been observed when solvents are present in the vial walls (increase $\leq$ 0.5% with toluene), but toluene and benzene, which evaporate only slowly from the vial walls, do cause increases in external standard counts of up to 13%. This is probably due to changes in γ-ray interaction characteristics, rather than increases in Cerenkov efficiency. The use of glass vials circumvents these problems at a 25% decrease in efficiency. No absorption problems have been encountered with aqueous solutions counted in polyethylene vials.

Chemical or physical phenomena which result in a non-homogeneous distribution of radioactivity in the vial will affect the efficiency. If an aqueous sample in polyethylene contains $^{32}P_i$ and all ions in the sample are below 10^{-4} M, efficiency increases with time because of adsorption of the phosphate to the vial (Figure 7). The increase can be up to 3% in 24 hours and up to 10% in 48 hours. In glass vials, efficiency decreases by no more than 2% in 48 hours, but decreases of less than 1% occur even in 0.1 M Na_2HPO_4, pH 7.5. In no case is there detectable efficiency change in 12 hours. This effect has been noted only when the labeled compound is orthophosphate, and the presence of most ions except Tris and HEPES at $\geq 10^{-4}$ M prevents the effect. It should also be noted that the molybdate complex of inorganic orthophosphate binds very strongly to polyethylene vials.

Figure 7 Change in Counting Efficiency with Time

0.1 ml aliquots of stock ^{32}P were mixed with 10 ml of the indicated phosphate buffer concentrations, counted immediately after mixing and at intervals afterward. The vials were stored in the refrigerated counting chamber. The data shows the % change in count rate relative to that predicted by normal decay and was computed for each individual vial according to:

$$\% = 100 \times \frac{\text{(observed cpm at time t)} - \text{(observed cpm at 0 time x decay correction)}}{\text{Observed cpm at 0 time x decay correction}}$$

▲: Polyethylene vials

■: Glass vials

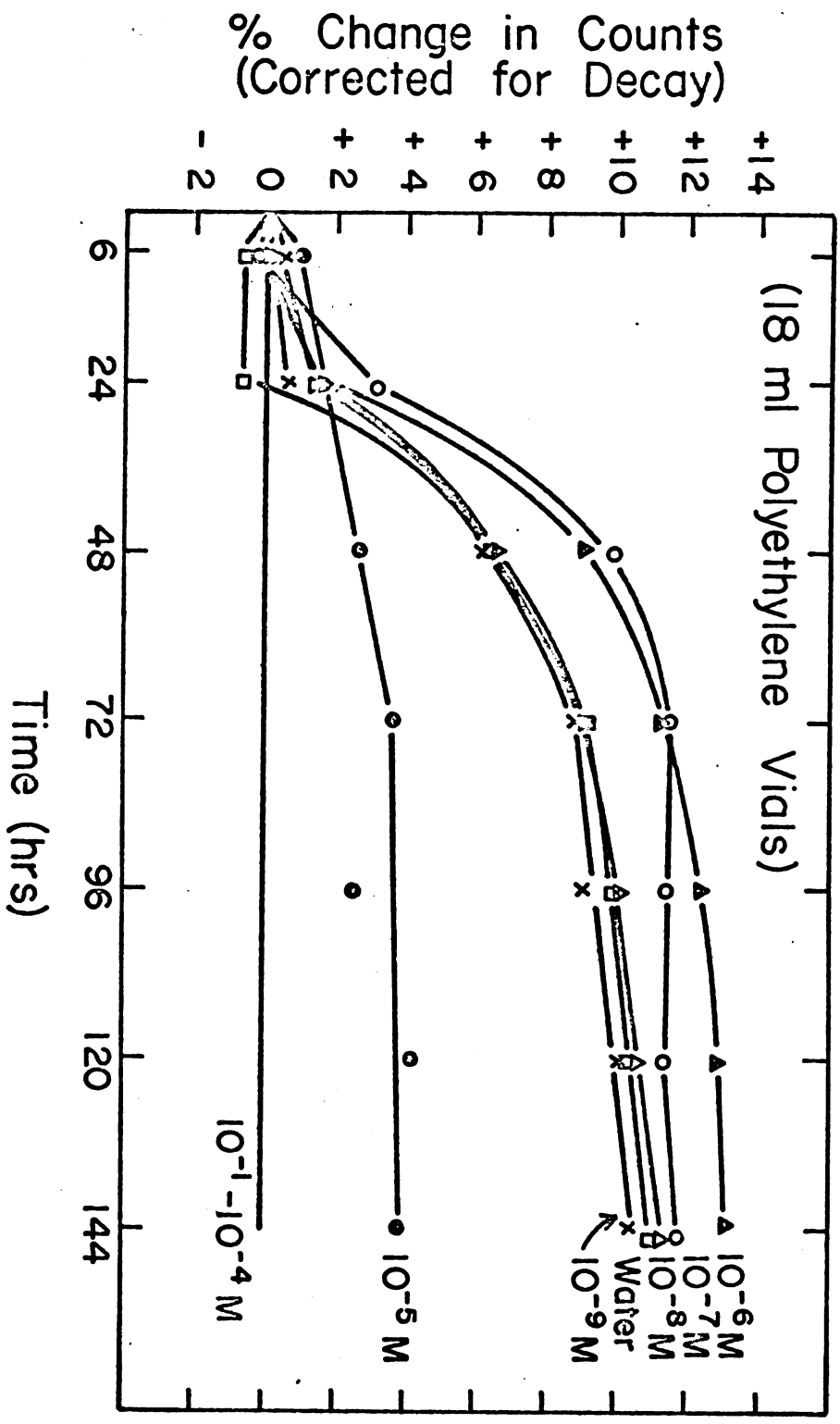
% Change in Counts
(Corrected for Decay)
- 2
0
+ 2
+ 4
+ 6
+ 8
+10
+12
+14
(18 ml Polyethylene Vials)
Time (hrs)
6
24
48
72
96
120
144
10^{-1}-10^{-4} M
10^{-5} M
10^{-9} M
Water
10^{-8} M
10^{-7} M
10^{-6} M

Figure 7a

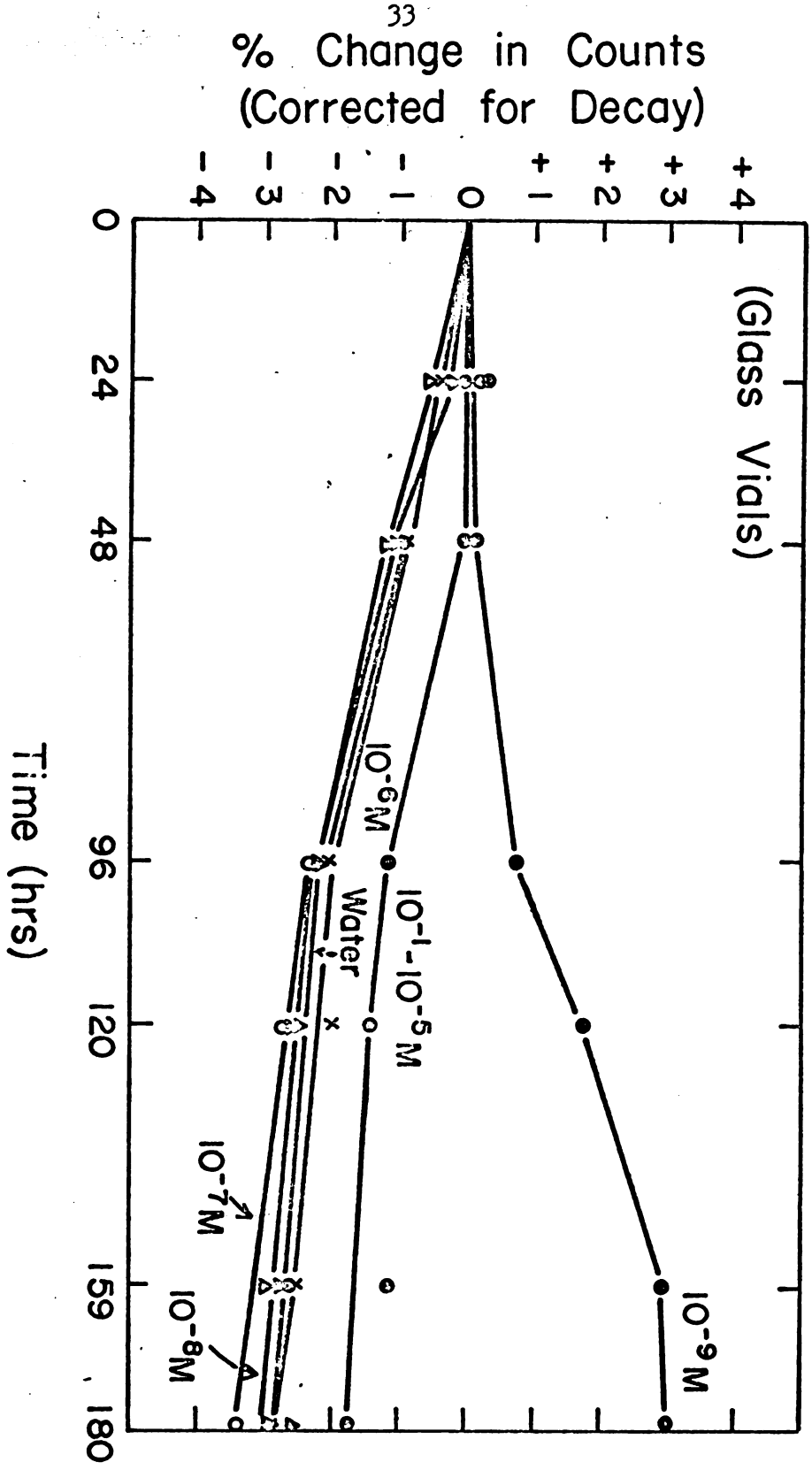
% Change in Counts
(Corrected for Decay)
-4
-3
-2
-1
0
+1
+2
+3
+4
0
24
48
96
120
159
180
Time (hrs)
(Glass Vials)
10-6M
10-1-10-5M
Water
10-7M
10-8M
10-9M

Figure 7b

Utility The following advantages of Cerenkov counting of ^{32}P have been pointed out by others:

1. No added scintillator is required.
2. Maximum counting volume is limited only by vial capacity.
3. Entire samples may be counted and recovered for further use.

Our results indicate that these advantages may be realized with essentially every ^{32}P-containing system of biological interest with the added convenience of constant instrument conditions. Though simple precautions must be taken to insure reproducibility of polyethylene vials, the accuracy and convenience of Cerenkov counting make it the method of choice for detection of ^{32}P.

BIBLIOGRAPHY

1. [illegible], V. E., Packard Technical Bulletin No. 16, January 1966.
2. [illegible], T., Anal. Biochem., [illegible], 70 (1968).
3. [illegible], R. F., and [illegible], E. G., Soil Sci. Amer. Proc., 32, 740 (1968).
4. STULEN, H. F., and JACKSON, [illegible], Int. J. Appl. Rad. Isotopes, 18, 857 (1967).
5. DAVIES, YARNOLD, Introduction to the Theory of Error, Addison Wesley, 1957.
6. MÜLLER, W., and [illegible], E., Anal. Biochem., 26, 483 (1968).
7. [illegible], F. (Editor), Scientific Tables, 6th Edition, Geigy Pharmaceuticals, Ardsley, New York, 1962.
8. JOHNSON, M. F., Appl. Biochem., 29, 348 (1969).
9. ROSS, H. F., Appl. Chem., 41, 1960 (1969).