SCINTILLATION COUNTING OF PAPER CHROMATOGRAMS

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There are many ways to determine the distribution of radioactivity in a paper chromatogram. Of these, direct counting by immersion of the paper section in a scintillation solvent has several advantages. Tedious (and perhaps incomplete) extraction of material is avoided; a satisfactory solvent for the labeled compound is unnecessary; counting efficiency is high; automatic recording equipment is available; and, since photomultiplier tubes have a very short resolving time, a very wide range of activities can be measured.

These obvious advantages have led to much interest in this technique as attested by at least three communications in the last eight months (1, 2, 3). Although none of these reports mentions pitfalls, a year of routine use of the technique has disclosed several sources of error. Since no two experimental systems are the same, we do not expect our experiences to be precisely duplicated in other laboratories. We report them here only in order to emphasize the necessity of extreme care if significant data are to be collected.

Our experimental system involved the determination of relative amounts of C^{14} radioactivity in compounds such as valine and valine hydroxamate. These compounds separate cleanly on a 7/8" x 5" strip of cationexchange paper (4) on which uncharged valine moves with the solvent front and the cationic valine hydroxamate is retained at the origin. Table I shows strikingly that the Packard Tri-Carb

Scintillation Counter is about ten times as efficient as the Nuclear-Chicago D-47 end window Geiger counter under these conditions.

TABLE I

	End window c.p.m.-bkg.	Packard[1,3] c.p.m.-bkg.	Packard[2,3] c.p.m.-bkg.
Thick paper[4]			
hydroxamate area	540	5529	4191
valine area	310	1575	1158
% hydroxamate[6]	64%	77%	78%
Thin paper[5]			
hydroxamate area	630	6031	5503
valine area	350	4113	4103
% hydroxamate[6]	64%	59%	63%

[1]strip oriented perpendicular to axis joining twin photo tubes.
[2]strip oriented parallel to axis joining twin photo tubes.
[3]Packard counter operated at Tap 6 (1080 volts) with the discriminator set at 7-80 volts, the optional setting for C^{14} homogeneous counting. The paper sections were suspended in a 5 dram vial, completely immersed in a toluene solution containing .03% POPOP and .3% POP.
[4]SA2 cation exchange paper containing about 50% Amberlite; weight about 17 mg/cm^2.
[5]SA1 cation exchange paper containing about 28% Amberlite; weight about 9 mg/cm^2.
[6]the mixture applied to the origin was 65% hydroxamate.

Although the scintillation technique was much more efficient than the end window technique, two serious flaws were observed. The orientation of the paper strip with respect to the photo tube influenced the efficiency and the apparent proportion of hydroxamate varied seriously with the paper thickness and orientation. Geiger and Wright (2) have considered the question of orientation and dismissed it as a serious factor. Of their three series only the glucose is actually on paper, the acetate being largely in solution. Glucose sample #4 shows a 20% variation with rotation.

In order to control the orientation problem the sections were folded 90° lengthwise or rolled into a closed cylinder form lengthwise. Table II shows how these measures reduced variation due

to orientation. The sample was the same as used in Table I.

TABLE II

Thick paper, Tap 6 7-80 volts	Rotated 0°	Folded 90° 45°	90°	180°	Cylinder 0°	45°	90°	180°
Hydroxamate	4694	4446	3838	3997	3592	3625	3450	3622
Amino acid	903	1568	1184	1375	972	1042	1001	982

The cylindrical form is clearly the most precise although some efficiency has been sacrificed. All further counting has been done with cylindrical sources.. Although the counting data are now relatively independent of the orientation, the apparent proportion of hydroxamate is 78% instead of 65%, as it should have been. This led us to examine the energy spectrum of the radiation from the two sections of paper. Figure 1 shows typical spectra obtained with a 10-40 volt window for valine and valine hydroxamate on thick or thin Amberlite SA paper.

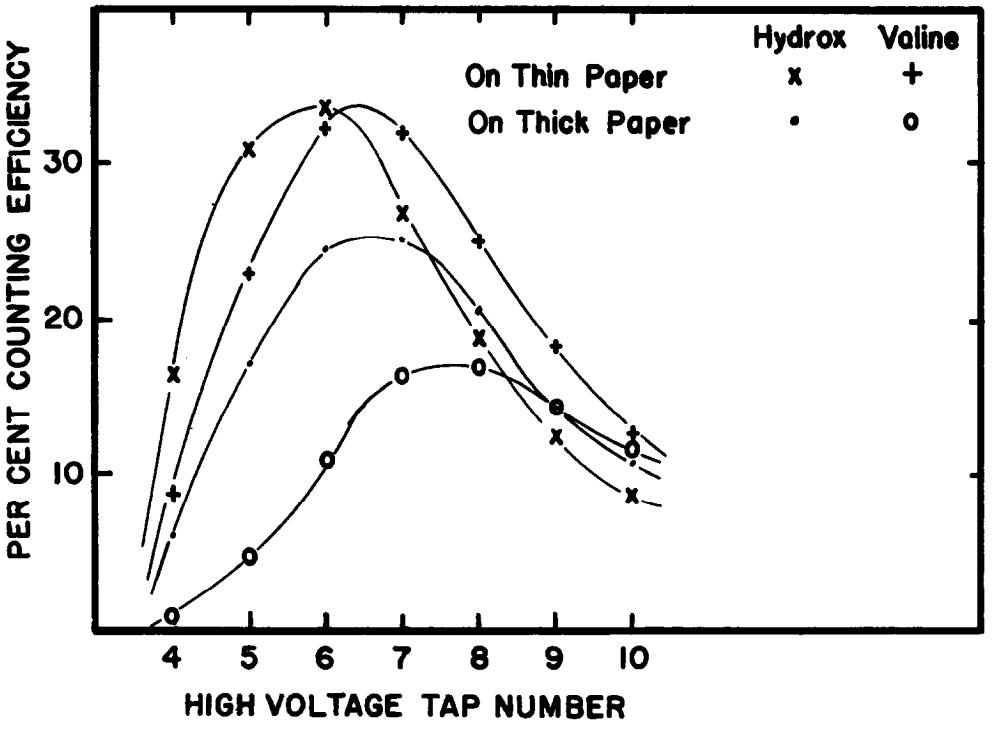

FIG. 1

One sees immediately that the spectra are strikingly different. If a mixture contained equal amounts of C^{14}-valine and C^{14}-valine hydroxamate, assay at Tap 4 on heavy paper would give an apparent ratio of 1 to 7; assay at Tap 10 on light paper a ratio of 3 to 2. If one wants to use uncorrected data one must select a high voltage tap and window setting at which both compounds are counted equally efficiently. This is obviously not the setting at which one particular compound is counted at maximal efficiency. For example, with the thick paper, Tap 9 gives the true ratio although neither the valine nor valine hydroxamate is counted most efficiently at this voltage. (Of course an acceptable alternative would be to count at a different tap and to multiply one value by a factor that converts to equivalent efficiency.)

We have found that if the paper strips were not evenly dried there is a variable lowering of efficiency of counting, that variations in the eluting buffer concentration cause variations in counting efficiency, that counting efficiency is independent of the presence or absence of substantial amounts of C^{12}-amino acids or protein.

Our present "standard" technique, subject to modification when this appears necessary, is to use 7/8" x 5" strips of thin Amberlite SA paper. 25 µl aliquots of our reaction mixture are applied to the origin (about 3/4" from one end). The enzyme is destroyed by 30 second steaming, the strip is dried and then suspended in a closed glass chamber while barely touching a .05 M, pH 7 sodium phosphate buffer (potassium is radioactive!). When capillarity brings the solution to within one-half inch of the top, the strip is removed and dried. Two inch sections at the origin and at the solvent front are rolled into a cylinder which just fits into a 10 mm. test tube filled with the scintillation solvent. The test tube is placed in the usual five dram vial, and counted in the Tri-

Carb counter at Tap 6, 10-50 volts. Occasional checks are made on the area between the two peaks to ensure the adequacy of the separation. Under these conditions determinations of hydroxamate or valine are accurate to within 3% over the range 100 c.p.m. to 80,000 c.p.m.

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