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LIQUID SCINTILLATION COUNTING OF SOLUTIONS CONTAINING CAROTENOIDS AND CHLOROPHYLLS

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LIQUID SCINTILLATION COUNTING OF SOLUTIONS CONTAINING CAROTENOIDS AND CHLOROPHYLLS.

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(Joint contribution from the Bio-Organic Chemistry Group, Lawrence Radiation Laboratory, University of California, Berkeley, and the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa)

ABSTRACT

Self-absorption in the liquid scintillation counting of solutions containing carotenoids and chlorophylls can be overcome by direct bleaching with chlorine. This simple technique restores most of the counting efficiency of colored solutions containing ^{3}H or ^{14}C, or both, and can be used to resolve these activities.

Bei der Scintillationszählung von Lösungen, die Carotinoide und Chlorophylle enthalten, kann Selbstabsorption vermieden werden durch direktes Bleichen mit Chlor. Dieses einfache Verfahren führt zur Wiederherstellung des grössten Teils der Zählwirksamkeit in ^{3}H oder ^{14}C bzw. beide Isotope enthaltenden gefärbten Lösungen und kann zur Trennung der beiden Aktivitäten verwendet werden.

The inherent advantages of liquid scintillation counting for the assay of ^{14}C or ^{3}H labeled carotenoids and chlorophylls are severely curtailed by color quenching.

Several techniques for the handling of color solutions have been developed (1,2,3,4).

For the routine and accurate assay of radioactivity in carotenoids and chlorophylls in these laboratories, however, a simpler method was needed. A significant variable which affects the counting efficiency of these substances has been studied and a direct and effective method for their assay by liquid scintillation counting has been developed. These data should be applicable to the assay of a wide variety of other colored substances by liquid scintillation counting.

EXPERIMENTAL

Equipment and Chemicals: Counting was done with an automatic Packard Tri-Carb liquid scintillation spectrometer Model 314. Discriminator gates were fixed at 10-50 and at 50-∞ . Crystalite screw cap 20 ml vials were used, filled to a 10 ml volume. Spectral data for characterization of pigments were obtained with a Cary recording spectrophotometer Model 14 and 10 mm internal path quartz cells. The scintillation solution used had the following composition: toluene, 2.0 liters; p-dioxane, 2.0 liters; ethanol, 1.2 liters; naphthalene, 260 gms; 2,5-diphenyloxazole (PPO), 26 gms; and 1,4-bis-(5-phenyl-2-oxazolyl)-benzene (POPOP), 0.5 gms. In designated instances, POPOP was omitted. Internal standards consisted of toluene-^{14}C or toluene-^{3}H, or both. Chlorophyll *a* from spinach leaves was prepared chromatographically on sugar columns by modifications of standard methods. Carotenoids, primarily β-carotene, were obtained chromatographically as a by-product of the preparation of chlorophyll *a*. The bleaching agent, chlorine

water, was prepared by bubbling chlorine gas to saturation through double glass-distilled water and stored in the dark at 2°C for use within 72 hours. For bleaching, 10 to 300 µl of chlorine water (depending on the concentration of the pigments) were added to the colored scintillation solution in the 20 ml vial, the cap screwed on tightly and the vial vigorously shaken for a few seconds. Carotenoids were bleached completely by this treatment. Chlorophylls acquired a light brown tint. The bleached chlorophyll color was metastable and a green component was regenerated after an interval of a few minutes to a few hours, depending on the concentration of the oxidant. Chlorine water could be added up to 500 µl to regenerate and extend the bleaching period without affecting the counting characteristics or phase stability of the solution in the cold. Addition of the chlorine bleach to these scintillation solutions did not affect significantly their counting characteristics, even after prolonged exposure.

RESULTS

The relation between pulse height and exciting particle energy producing the pulse is a function of the amplification of the instrument. This gain depends on the high voltage applied to the photomultipliers in the coincidence circuit. In the Packard Tri-Carb scintillation spectrometer Model 314, this parameter can be changed from 680 volts for high voltage tap 1 (HVT-1) to 1450 volts for high voltage tap 10 (HVT-10) in successive increments of approximately 85 volts per tap. Holding other variables constant, the counting efficiency of various solutions containing carotenoids and/or chlorophylls were measured as a function of the HVT. These results are plotted in Figures I to IV.

Figure I shows the effect of chlorophyll at several concentrations and of carotenes, on the scintillation counting of a standard 2.46 x 10^4 dpm toluene-^{14}C sample. It can be seen that the counting efficiency appears as an inverse function of concentration. There is also a concomittant shift of optimal HVT.

In Figure II a standard 1.86 x 10^5 dpm toluene-^{3}H sample was exposed to the same quenching solutions used in Figure I. In this case, there was a much greater loss of counting efficiency than was observed with ^{14}C, but there was no significant shift in the HVT. Thus, the overall effect of chlorophyll and carotenes solution was to render a simultaneous assay of ^{14}C and ^{3}H less precise, not only through a marked decrease in counting efficiency, but also by narrowing the HVT gap necessary to resolve these two activities.

It should be noted that in experiments involving solutions of both chlorophyll and carotenes, the effects just described above were quantitatively additive. Also, when POPOP was omitted from the scintillation solution, a decrease in efficiency not greater than 0.6% was observed in all instances studied.

The effect of the chlorine bleach treatment on the highest concentration of chlorophyll (4 mg per liter) and carotenes (1 mg per liter) examined above, is shown in Figure III for ^{14}C and in Figure IV for ^{3}H. In the case of ^{14}C (Figure III) the bleaching treatment resulted in a nearly complete restoration of counting efficiency and optimal HVT of the control solution. Bleaching of the chlorophyll solution resulted in a less dramatic, but marked improvement in both counting efficiency and HVT, which amply justified the use of this technique. Since there was not a serious HVT shift

problem with ^{3}H, the main advantage of the bleaching treatment was to restore most of the counting efficiency of carotene solution, and a significant portion of it for the chlorophyll solution. Also, the low counting efficiency observed with these colored solutions appears to be due almost entirely to self-absorption which decreases pulse height. The discrimination circuit rejects the low energy scintillations thus requiring an increased high voltage on the photomultiplier for optimum counting efficiency. This effect is sufficiently large in the case of carbon-14 to shift the HVT to a higher value. Thus, these problems are either considerably minimized or entirely eliminated with the direct bleaching technique.

FOOTNOTE

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BIBLIOGRAPHY

1. Baillie, L.A., Intern. J. Appl. Radiation and Isotopes,,

2. Herberg, R.J., Anal. Chem. 32, 1468 (1960).

3. Domer, F.R., Hayes, F.N., Nucleonics 18, 100 (1960).

4. Eisenberg, F., Jr., "Liquid Scintillation Counting", p. 123, Pergamon Press, New York, 1958. Quinn, V.P. loc. cit. p. 176.

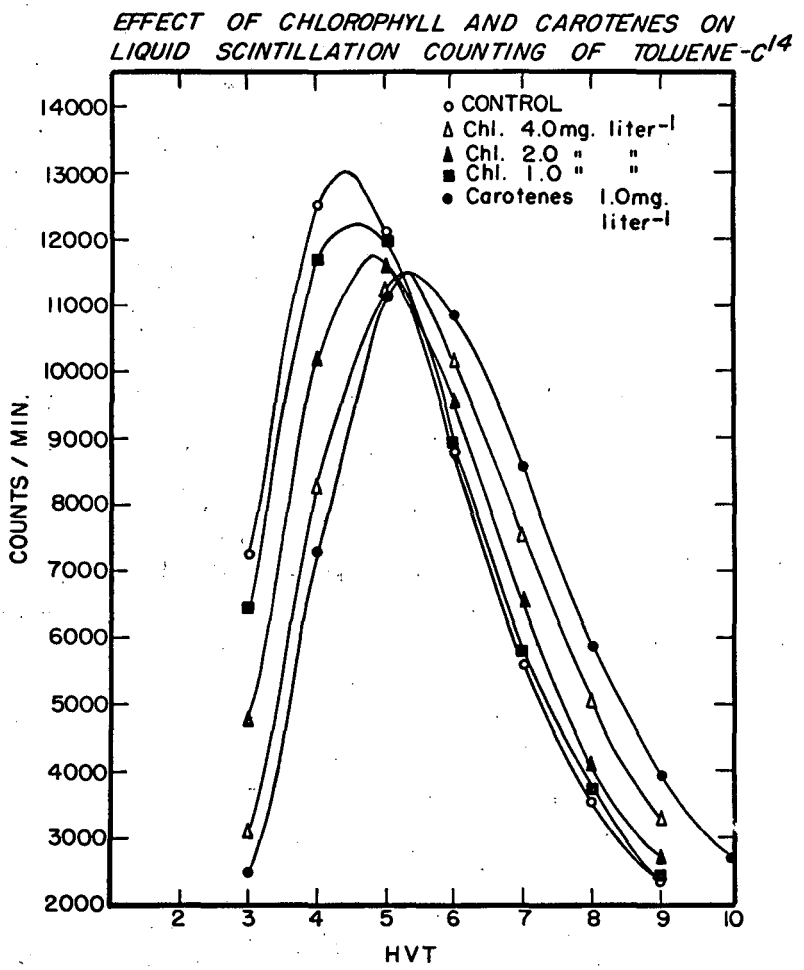
EFFECT OF CHLOROPHYLL AND CAROTENES ON
LIQUID SCINTILLATION COUNTING OF TOLUENE-C14
o CONTROL
Δ Chl. 4.0mg. liter-1
▲ Chl. 2.0 " "
■ Chl. 1.0 " "
● Carotenes 1.0mg. liter-1
COUNTS / MIN.
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
2
3
4
5
6
7
8
9
10
HVT

Figure I.

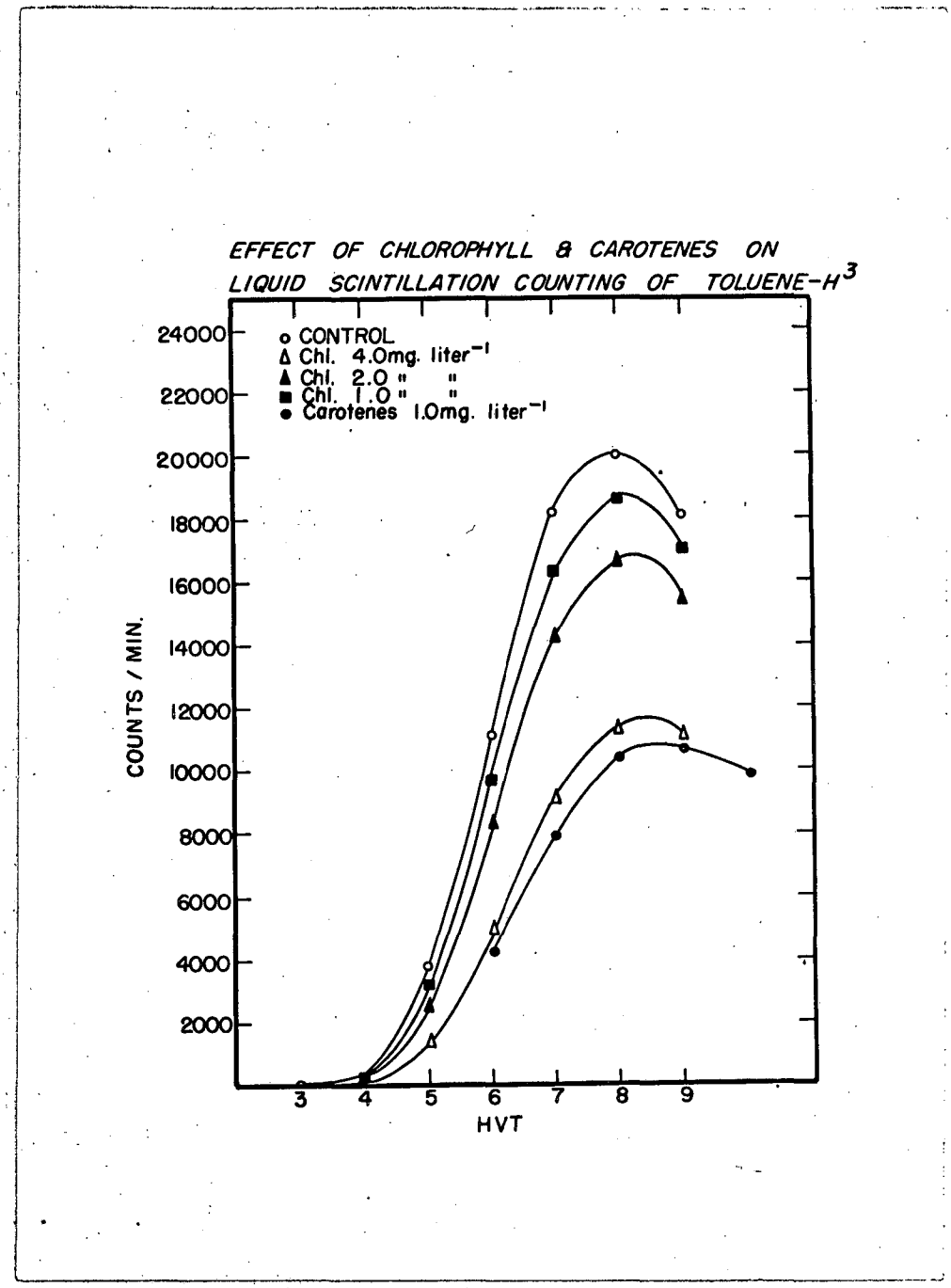
EFFECT OF CHLOROPHYLL & CAROTENES ON
LIQUID SCINTILLATION COUNTING OF TOLUENE-H3
o CONTROL
Δ Chl. 4.0mg. liter-1
▲ Chl. 2.0 " "
■ Chl. 1.0 " "
● Carotenes 1.0mg. liter-1
COUNTS / MIN.
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
3
4
5
6
7
8
9
HVT

Figure II.

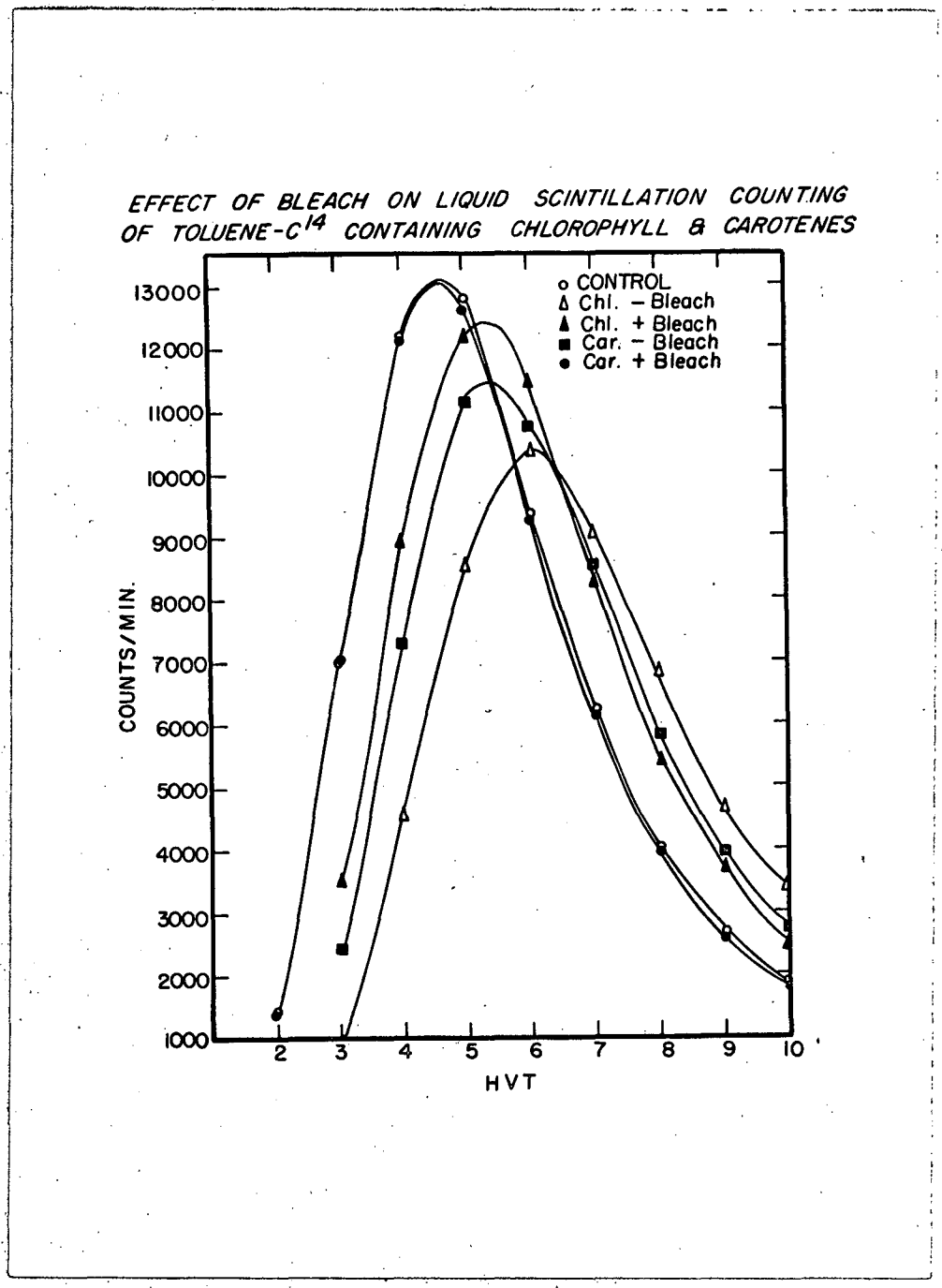
EFFECT OF BLEACH ON LIQUID SCINTILLATION COUNTING
OF TOLUENE-C14 CONTAINING CHLOROPHYLL & CAROTENES
o CONTROL
Δ Chl. – Bleach
▲ Chl. + Bleach
■ Car. – Bleach
● Car. + Bleach
COUNTS/MIN.
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
2
3
4
5
6
7
8
9
10
HVT

Figure III.

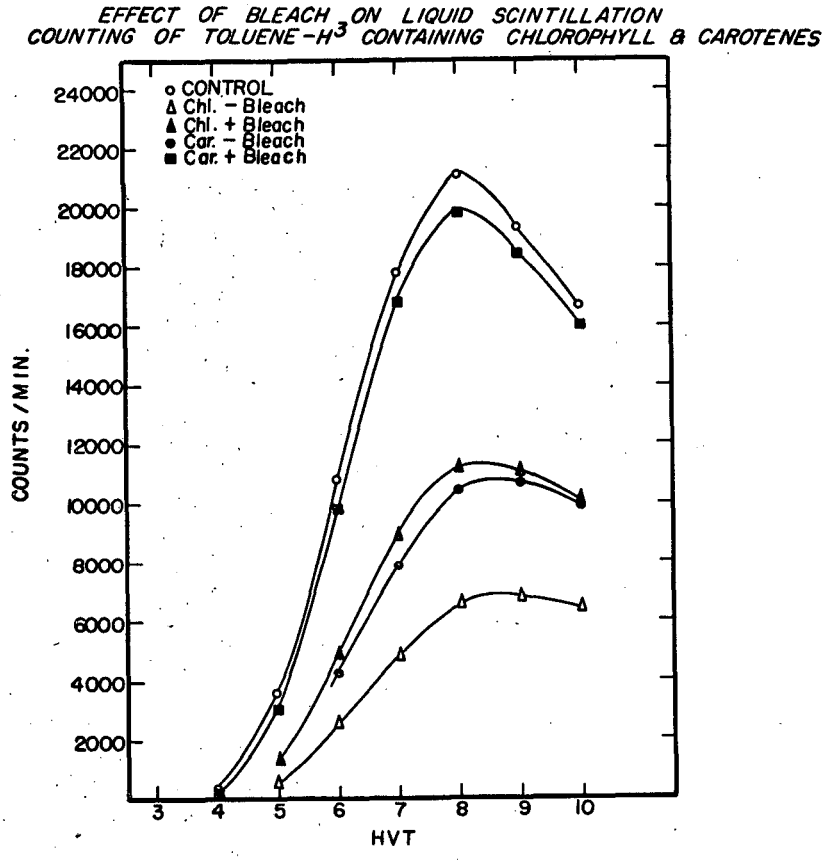
EFFECT OF BLEACH ON LIQUID SCINTILLATION
COUNTING OF TOLUENE-H3 CONTAINING CHLOROPHYLL & CAROTENES
o CONTROL
Δ Chl. – Bleach
▲ Chl. + Bleach
● Car. – Bleach
■ Car. + Bleach
COUNTS / MIN.
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
3
4
5
6
7
8
9
10
HVT

Figure IV.