

## CORRECTION FOR QUENCHING IN LIQUID SCINTILLATION

### COUNTING OF HOMOGENEOUS SAMPLES

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In liquid scintillation counting of homogeneous solution samples, the organic phosphor is mixed with a small amount of the radioactive solution to be assayed. The introduction of foreign material into the phosphor system frequently causes a diminution of the maximum light output that is obtained from radiation energy dissipated in the system by the beta particles. In theory, the radiation energy is transferred molecularly through the solvent to the phosphor which becomes excited and then de-excites with the emission of light as fluorescence (1,2). When non-fluorescent materials are present, degradation of the excitation energy may take place without emission of light. This quenching of fluorescence reduces the counting efficiency of the liquid scintillation system, and leads to erroneous results in assaying the radioactive samples if the extent of quenching is not considered.

Correction for the loss of sample counts due to quenching may be achieved either by an internal standard method (3-6), by the extrapolation method (7,8), or by comparing the pulse-height distribution in terms of the ratio of counts in two channels in the liquid scintillation spectrometer (3). In the latter method the two channels must be especially adjusted for this purpose. This report is concerned with the merits and shortcomings of the first two methods.

The internal standard method, which is widely used for correcting quenching loss, involves the addition of a radioactive compound to the counted sample, followed by a recount; the increment in counts thus observed is attributed to the added internal standard which is presumably being quenched to the same extent as the sample. From the ratio of the known counts of the added standard to the observed increment in counts

in the sample, the true counting rate of the sample may be computed. The accuracy and precision of this method under varied conditions have not been evaluated despite the earlier observation by Davidson and Feigelson (3) that the internal standard technique is useful in calculating a correction for slight quenching but is of doubtful validity when the quenching correction is large. Whisman and his co-workers (6) applied the internal standard technique to the radioassay of some tritiated hydrocarbons and studied the precision of the method but their evaluation did not take into consideration the conditions of varying degrees of quenching under which the internal standard method may not apply.

The extrapolation method consists of radioassaying the sample at several different sample concentrations in the liquid scintillator. Since quenching is concentration dependent (9), decreasing the sample concentration causes a corresponding increase in the counting efficiency of the system or the counts observed per unit sample. If the counting rate per unit weight (or volume) of the sample at each sample concentration is plotted against the corresponding concentration on semi-log paper, a linear relationship results. By extrapolation to zero sample concentration where, theoretically, no quenching takes place, the true activity of the sample per unit weight (or volume) is obtained. The validity of this method in application for quenching correction has been reported earlier (7,8).

Since the extrapolation method uses many points for extrapolation thereby giving proper weighting to each observation, it is therefore inherently more accurate than the internal standard method which relies on a single observation and is consequently more liable to experimental error. In Table 1, the merits and shortcomings of the two methods, based upon the results of the present study, are listed.

The precision of the internal standard method versus the extrapolation method was studied under comparable but varying conditions of quenching with samples containing succinic-2,3-C<sub>2</sub><sup>14</sup> acid and tritiated methyl stearate of high specific activity. The method employed is as follows:

Azobenzene, Apiezon wax, iodine and para-nitroaniline were dissolved in toluene to a concentration of 315.7, 52.1, 67.6, and 50.3 mg. per ml. respectively, and the solutions were used as quenching agents. A known number of counts of C-14 and H-3 was added separately to each solution of the quenching agents and the solution was then assayed by the extrapolation method using sample concentrations ranging from 10

Table 1

## COMPARISON OF MERITS AND SHORTCOMINGS

| <u>Internal Standard Method</u>   | <u>vs.</u> | <u>Extrapolation Method</u>   |
|---|------------|---|
| 1. Single counting sample required.   |            | 1. Multiple counting samples required.  |
| 2. Results more prone to experimental error.                                      |            | 2. Results plotted according to equation.<br><br>$S_a = S_0 \exp. (-qC)^*$<br><br>thereby leading to more accurate weighting of each observation. |
| 3. Selection of an adequate radioactive compound for internal standard necessary. |            | 3. No internal standard required.   |
| 4. Accuracy affected by the concentration of quencher present.                    |            | 4. Applicable to high concentration of quenchers, if the above equation is obeyed.  |
| 5. Accuracy affected by the level of radioactivity added as internal standard.    |            | 5. Quenching constant, $q$ , is independent of specific activity of sample.   |

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\* $S_a$  is the apparent specific activity of the sample at concentration  $C$ ;  $S_0$ , the specific activity of the sample in the absence of quenching;  $q$ , the quenching constant, equal to  $0.693/C_{1/2}$ , where  $C_{1/2}$  is the sample concentration that will reduce the counts to half of initial value by quenching. See Ref. (3).

microliters to 0.5 ml. in 10 ml. of a mixed liquid scintillator.\* Upon completion of counting, a known number of counts of C-14 or H-3 in 50 microliters of toluene was added as internal standard to the counted samples containing the respective isotopes and the samples were then re-counted. All the samples were integral-counted in 10- $\infty$  channel in a Tri-Carb automatic liquid scintillation spectrometer\*\* at the balance point repeatedly for several 10-minute periods. This yields a statistical accuracy of 0.5% or better. The various background samples were counted at the same settings at 10-minute intervals.

In the experiments studying the effect of quencher concentration and likewise the number of counts added as internal standard, four replicate samples were employed. In this manner, the accuracy and validity of quenching factor was established.

### Results and Discussion

Figures 1, 2, 3 and 4 are displays of the counting results of sample containing azobenzene, Apiezon wax, iodine and para-nitroaniline as quenchers, plotted according to the extrapolation method. Figure 2 and 4 show on an expanded scale the initial portion of each curve; the lines are regression lines for points corresponding to sample concentration of 10, 20, 40, 60, 80 and 100 microliters in 10 ml. scintillator, and the intercepts represent the counting rate of the sample in the absence of quenching. The average numerical value for the calculated intercepts from these four sets of samples are given in Table 2.

It can be seen that the plots for C-14 samples containing azobenzene and para-nitroaniline exhibit a linear relationship at low concentrations of the quencher but deviate significantly from that relationship at high concentrations; this deviation may seriously limit the usefulness of the extrapolation method in assaying radioactive solutions containing high concentrations of strong quenchers. In contrast, the plots of C-14 samples containing Apiezon wax and iodine as coloring and quenching agents remain linear throughout the concentration used. This discrepancy in the behavior of these sets of samples may be explained on the basis that in addition to fluorescence quenching there is also an "optical" quenching as a result of the coloring of the solution which may reduce the transmission of light produced in the system disproportionately from that due to fluorescence quenching alone.

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\* The mixed scintillator consists of a mixture of equal volumes of the toluene scintillator (3.0 g./l.) and the dioxane scintillator (7.0g. PPO, 0.05 g. POPOP, and 50 g. naphthalene to 1 l. with purified dioxane) (10).

\*\*Manufactured by the Packard Instrument Company, LaGrange, Ill.

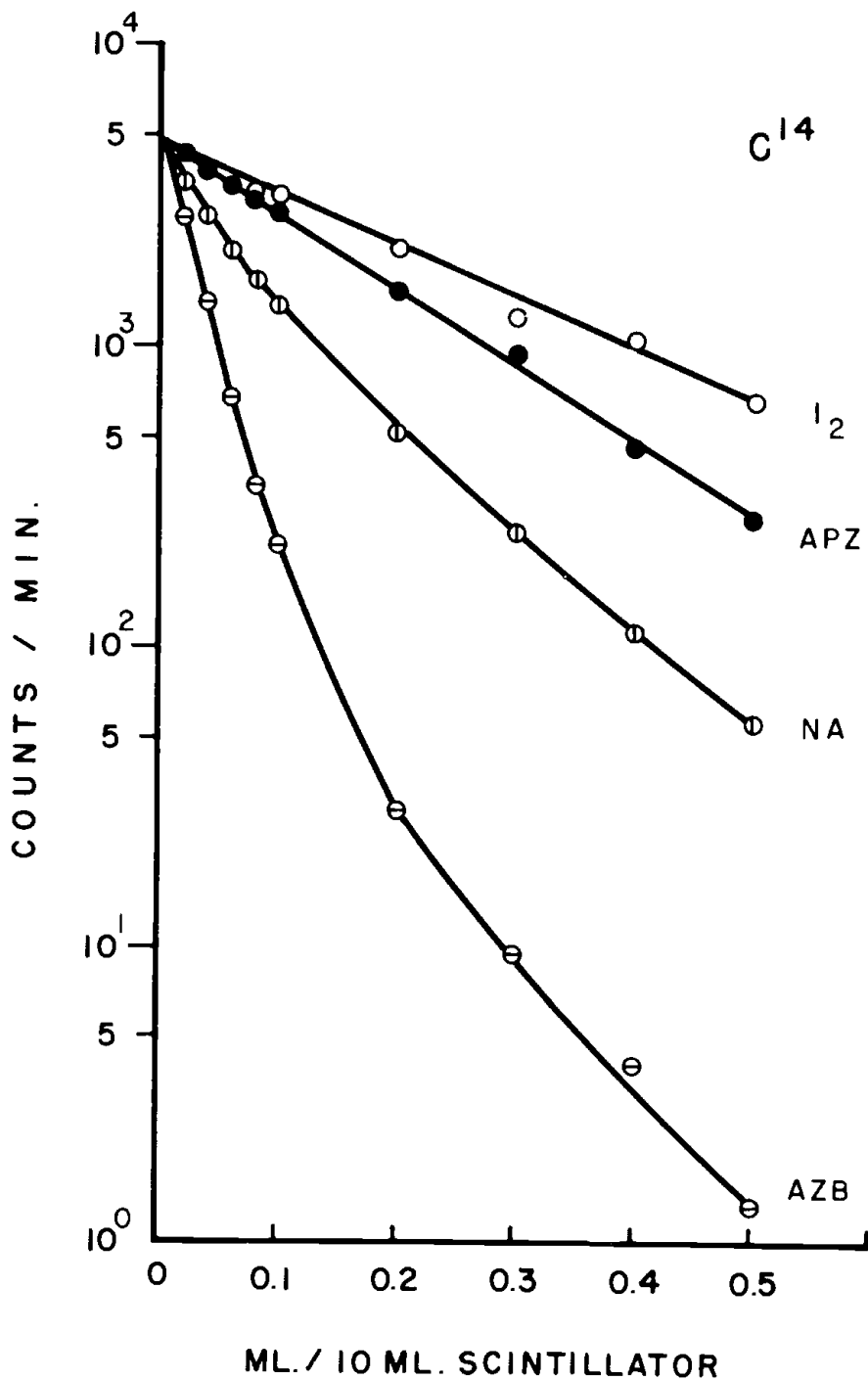


Fig. 1. Apparent specific activity versus sample concentration for  $C^{14}$  samples (APZ for Apiezon wax; AZB for azobenzene; NA for para-nitroaniline, and I<sub>2</sub> for iodine).

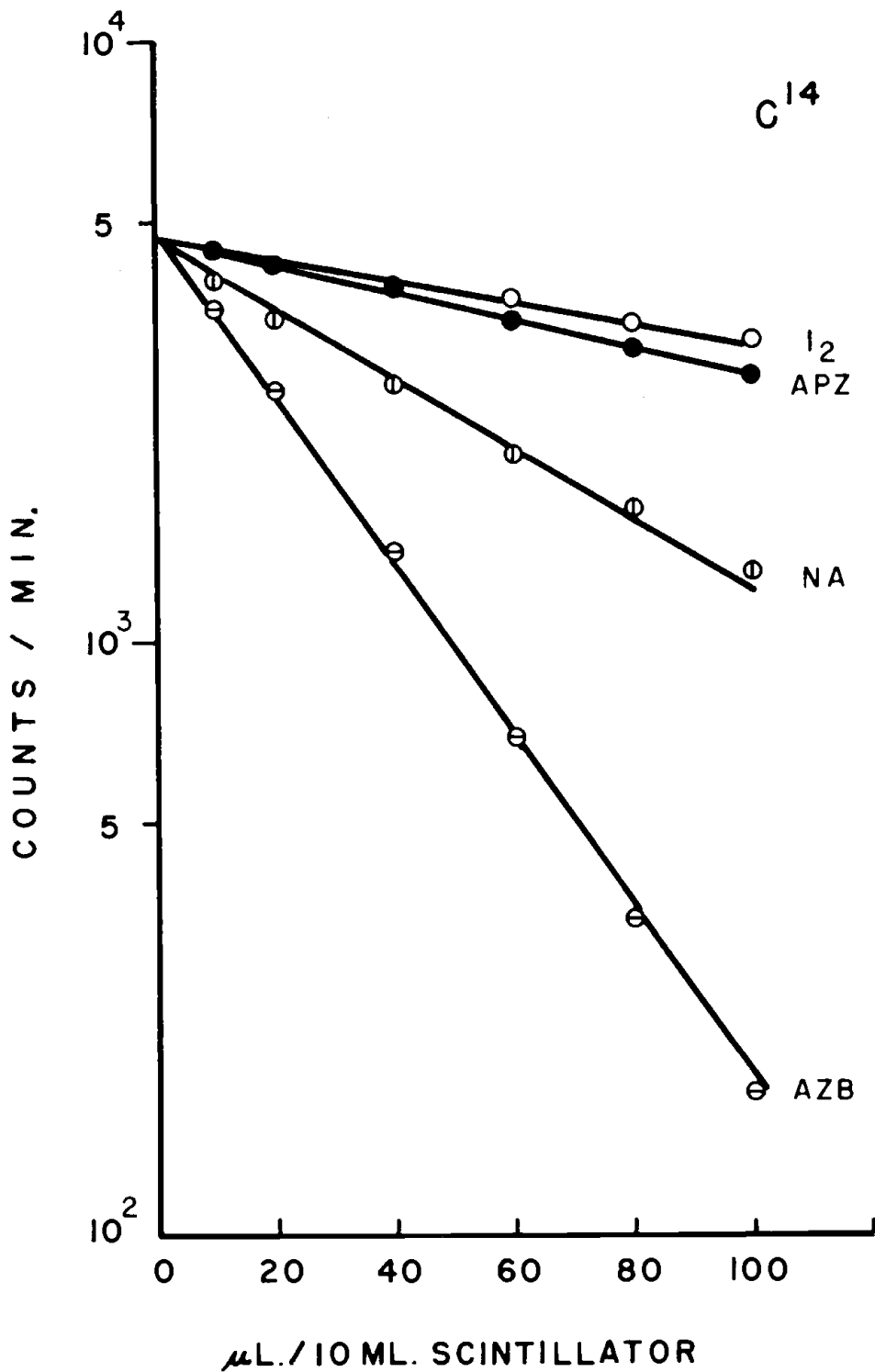


Fig. 2. Replot of Fig. 1 on expanded concentration scale.

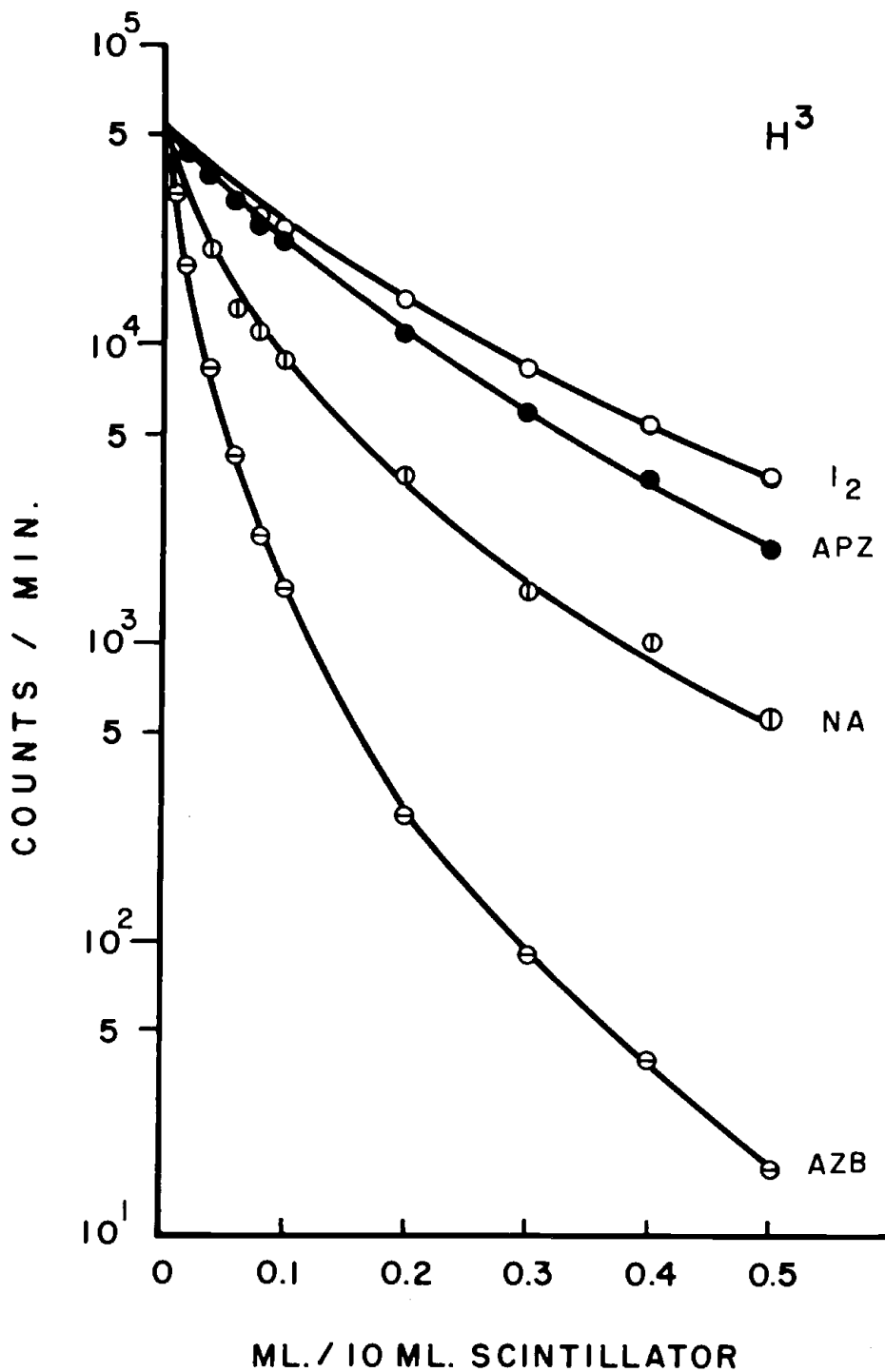


Fig. 3. Apparent specific activity versus sample concentration for  $H^3$  samples.

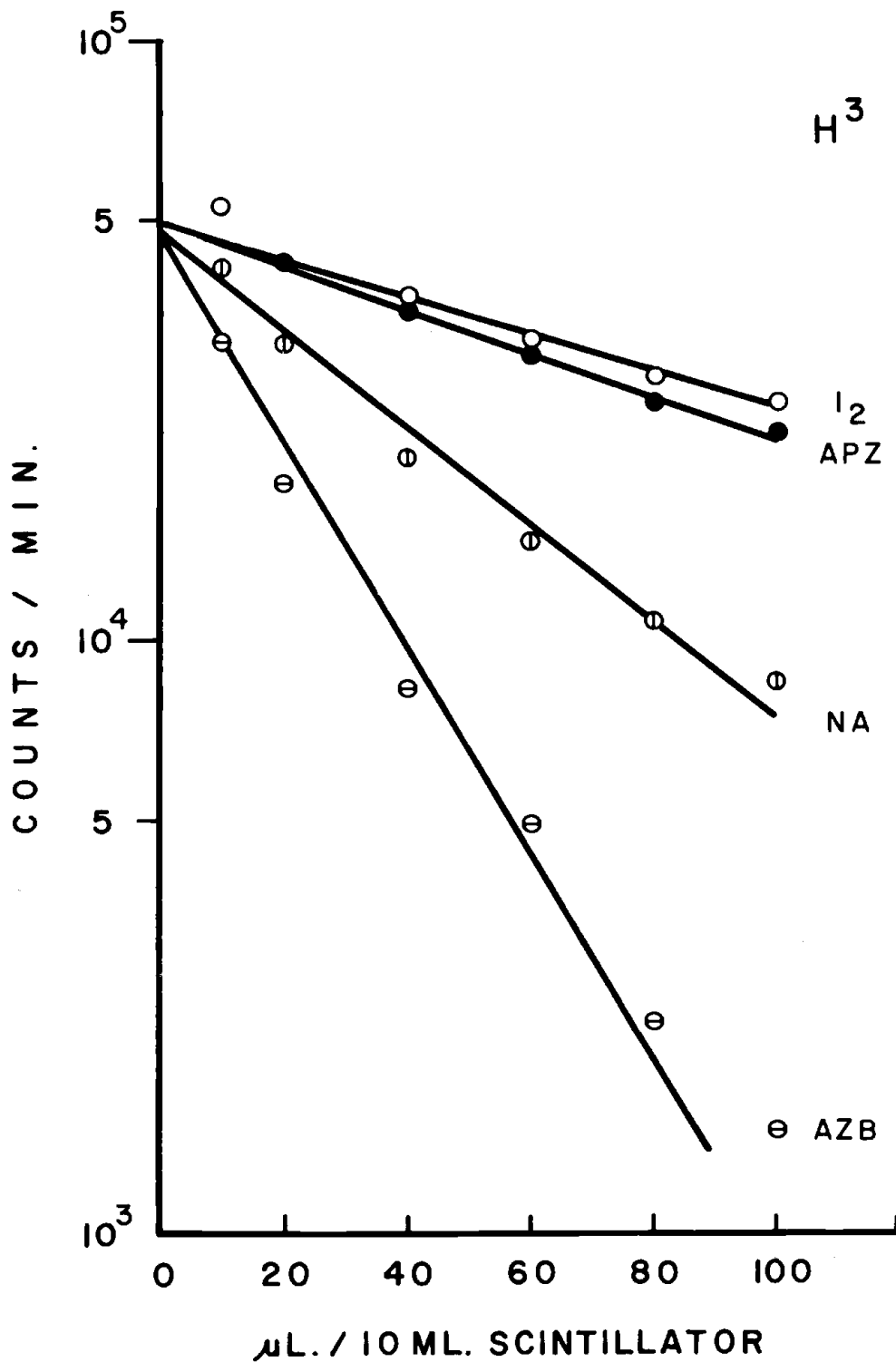


Fig. 4. Replot of Fig. 3 on expanded concentration scale.

Table 2  
COMPARISON OF ACCURACY OF RESULTS

|                                       | C <sup>14</sup>                   | H <sup>3</sup> |
|---------------------------------------|-----------------------------------|----------------|
|                                       | Counts / 10 min. / 0.1 ml. sample |                |
| True value                            | 46,400                            | 511,094        |
| Value obtained by:                    |                                   |                |
| 1) Internal standard method           |                                   |                |
| Apiezon wax                           | 48,177 ± 3%*                      | 563,674 ± 10%  |
| Azobenzene                            | 46,358 ± 13%                      | 581,133 ± 7%   |
| Iodine                                | 46,339 ± 8%                       | 451,829 ± 18%  |
| <u>para</u> -Nitroaniline             | 46,750 ± 4%                       | 543,180 ± 11%  |
| Av.                                   | 46,906                            | 509,979        |
| 2) Extrapolation method               |                                   |                |
| Av. intercepts of<br>regression lines | 47,415                            | 497,633        |

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$$*\text{Standard deviation} = \sqrt{\frac{n \sum X^2 - (\sum X)^2}{n(n-1)}}$$


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Another plausible explanation for the discrepancy is that at high sample concentrations, the solution may deviate from an ideal solution in that the solute molecules tend to associate thereby decreasing the effective concentration of the quencher present; consequently, a positive deviation from the predicted straight line as evidenced in Figures 1 and 3 results.

Apparently, this deviation is energy dependent and varies inversely with the intensity of light produced in the system, because under identical conditions, the plots for H-3 samples were found to deviate more from the linear relationship than the C-14 samples.

The precision of the extrapolation method in determining the sample counts in the absence of quenching is 2.1% for C-14 samples and 2.5% for H-3 samples. The variation among the values of the intercept of the regression line from the four sets of samples is within 1.7% and 7.3% of the standard deviation of the mean for samples containing C-14 and H-3 respectively.

When all the counted samples were "spiked" with an internal standard and recounted, the increment in counts in each sample was used to compute the counts initially present without quenching. The results obtained from samples containing 10, 20, 40, 60, 80 and 100 microliters of azobenzene, Apiezon wax, iodine, and para-nitroaniline in 10 ml. scintillator are given in Table 2. It may be noted that the values obtained from C-14 samples were identical within the experimental error to the counts added initially while those obtained from H-3 samples showed a greater dispersion of the data and were approximately 9% higher than the true value. The value from H-3 samples containing iodine was not included in this computation.

When the factor derived from samples containing concentration of quenchers in excess of 100 microliters in 10 ml. scintillator was used for quenching correction, higher values of sample count than that added initially were obtained. In general, the deviation from the true sample count becomes greater as the concentration of the quencher increases, with the exception of H-3 samples containing iodine which showed a trend in the direction of negative deviation.

The dependence of the accuracy of radioassay by internal standard method upon the concentration of the quencher present was studied using C-14 samples containing different quantities of azobenzene in 10 ml. scintillator. The results are given in Table 3. Ratio of the sample

Table 3

ACCURACY VS. CONCENTRATION OF QUENCHER IN SCINTILLATOR

|  |                  |
|--|------------------|
| Counts initially present, $C_0$ :                                    | 59,187 c/10 min. |
| Counts added as internal standard<br>in 0.05 ml. toluene, $C_{IS}$ : | 39,212           |

| Mg. of azobenzene in<br>0.1 ml. toluene added<br>to 10 ml. scintillator | Ratio: counts<br>remaining after<br>quenching to $C_0$ | Ratio: counts<br>derived from<br>correction to $C_0$ |
|---|--|--|
| 3.95  | 0.032  | 1.85   |
| 1.58  | 0.264  | 1.96   |
| 0.789   | 0.526  | 1.15   |
| 0.158   | 0.888  | 1.03   |

counts derived from quenching correction to the sample counts initially added shows a significant deviation from unity as the concentration of azobenzene increases. This finding confirms the observation of Davidson and Feigelson (3) that the internal standard technique is valid only under conditions of minimal quenching.

The level of activity added as internal standard may also affect the accuracy of the factor for quenching correction. Table 4 shows the

Table 4

ACCURACY VS. LEVEL OF RADIOACTIVITY ADDED AS INTERNAL STANDARD

|   |                          |  |
|---|--------------------------|--|
| Counts initially present, $C_0$ :   | 82,219                   | c/10 min.                                      |
| Counts remaining after addition of 0.394 mg. azobenzene in 0.1 ml. toluene: | 59,871                   |  |
| Counts added as internal standard in 0.05 ml. toluene, $C_{IS}$             | Ratio: $C_{IS}$ to $C_0$ | Ratio: counts derived from correction to $C_0$ |
| 998,961   | 12.4                     | 1.03   |
| 212,858   | 2.59                     | 1.03   |
| 29,201  | 0.356                    | 1.06   |
| 19,166  | 0.233                    | 1.25   |
| 5,790   | 0.07                     | 1.45   |

results of a study in which internal standards of varying levels of activity from 0.07 to 12.4 times the initially added sample counts were used. The ratio of the sample counts obtained by correction to that initially present also showed a deviation from unity. Conceivably, the extent of this deviation is dependent upon the concentration of the quencher. It is clear that under the quenching conditions prevailing in this experiment, in order to determine accurately a sample count in the absence of quenching by correction, an internal standard of comparable or higher activity than that of the true sample counts should be added.

Background counts

Domer and Hayes (11) observed that background counts are quenched differently from the sample counts. Therefore, the accuracy in assaying weakly active, strongly quenched, colored samples such as those

containing biological specimens depends largely upon an exact knowledge of the background counts of the sample. Herberg (12) measured the background counts of a colored sample accurately by using a colorimetric technique. Under varying conditions of fluorescence and "optical" quenching, the individual sample background counts may vary; but in cases of severe quenching, the unquenched portion of the background counts tends to approach a limiting value.

The composition of background counts in liquid scintillation counting was reported (8) to be attributable to 1) chance coincidence, 2) reciprocal sensitization of the photocathodes of the multiplier phototubes, 3) potassium-40 in glass, 4) Cherenkov radiation, 5) cosmic radiation, and 6) environmental radioactivity. In a clear, transparent sample containing quenchers, the fraction of the total background counts of the sample due to cosmic radiation and environmental radioactivity will be quenched proportionately as the sample counts, provided that the background counts have a comparable pulse-height distribution as the sample counts; whereas, the remaining fraction of the background counts due to chance coincidence, reciprocal sensitization of photocathodes, potassium-40 in glass, and Cherenkov radiation will not be quenched. However, in a colored quenching sample, in addition to fluorescence quenching, there is also an attendant "optical" quenching, which diminishes the transmission of light from Cherenkov radiation and potassium-40 in glass thereby reducing the background counts of the sample to a value, attributable mainly to chance coincidence. The results of a series of samples studied are given in Table 5.

#### Fluorescence Quenching in Liquid Scintillator vs Sources of Excitation

In order to determine whether the fluorescence excited by ionizing radiation is quenched similarly as that excited by ultraviolet light in the presence of a quenching agent in another approach to quenching correction in liquid scintillation counting, scintillator solutions containing varying amounts of quenchers were measured in an Aminco fluorospectrophotometer. For the particular composition of the mixed scintillator solution used in this experiment, the exciting wavelength used was found to be 3900 Å., and the wavelength of the main band of the fluorescence spectrum was 4200 Å. When the reciprocal of the fluorescence readings at 4200 Å was plotted against the concentration of the quencher on semi-log paper, a linear relationship was obtained. Scintillator solutions of identical composition were also used to count C-14 or H-3, and the ratio of the sample counts added initially to that observed was similarly plotted against the concentration of the quencher. Straight or nearly straight lines as shown in Figure 5 were obtained.

Liquid scintillators containing ferric chloride or Apiezon wax as quenching agents showed comparable quenching of fluorescence in solution,

Table 5

## BACKGROUND COUNTINGS

| No.                           | Sample  | Settings           |                |
|-------------------------------|---|--------------------|----------------|
|                               |   | C <sup>14</sup>    | H <sup>3</sup> |
|                               |   | Counts per 10 min. |                |
| (Channel width: 10 - ∞ volts) |   |                    |                |
| 1                             | Dark vial   | 63 ± 24*           | 148 ± 33       |
| 2                             | Blank sample well   | 129 ± 20           | 405 ± 56       |
| 3                             | Empty glass vial  | 141 ± 17           | 612 ± 53       |
| 4                             | Toluene**   | 209 ± 19           | 859 ± 50       |
| 5                             | Mixed scintillator**                                      | 499 ± 21           | 1131 ± 63      |
| 6                             | FeCl <sub>3</sub> solution I <sup>+</sup>                 | 93 ± 19            | 287 ± 47       |
| 7                             | FeCl <sub>3</sub> solution II <sup>+</sup>                | 87 ± 20            | 323 ± 57       |
| 8                             | FeCl <sub>3</sub> -scintillator solution I <sup>++</sup>  | 110 ± 32           | 379 ± 45       |
| 9                             | FeCl <sub>3</sub> -scintillator solution II <sup>++</sup> | 130 ± 22           | 505 ± 73       |

\* Average of at least 16 readings. Standard deviation calculated by

$$\sqrt{\frac{n \sum X^2 - (\sum X)^2}{n(n-1)}}$$

\*\*10 ml. solution, not de-oxygenated.

+ I and II containing 340.4 mg. and 22.4 mg. FeCl<sub>3</sub>·6H<sub>2</sub>O, respectively per 10 ml. solution of equal volumes of para-dioxane and toluene.

++ I and II containing 123.5 mg. and 24.7 mg. FeCl<sub>3</sub>·6H<sub>2</sub>O, respectively, per 10 ml. mixed scintillator.

irrespective of the means of excitation, although the slope of the two lines differed slightly. With azobenzene as a quencher, quenching is approximately four times more severe with ionizing radiation as the exciting source than with uv light; whereas, with para-nitroaniline, the opposite is true. In liquid scintillators containing increasing amounts of hyamine hydroxide or phenyl isothiocyanate, the intensity of fluorescence at 4200 Å remained practically unaltered while these same solutions showed strong quenching properties as counting systems.

These interesting but fragmentary observations serve to emphasize again the complexity of the energy transfer mechanism in liquid scintillation systems. Only when this mechanism is completely understood can a more accurate correction for quenching in counting samples be made.

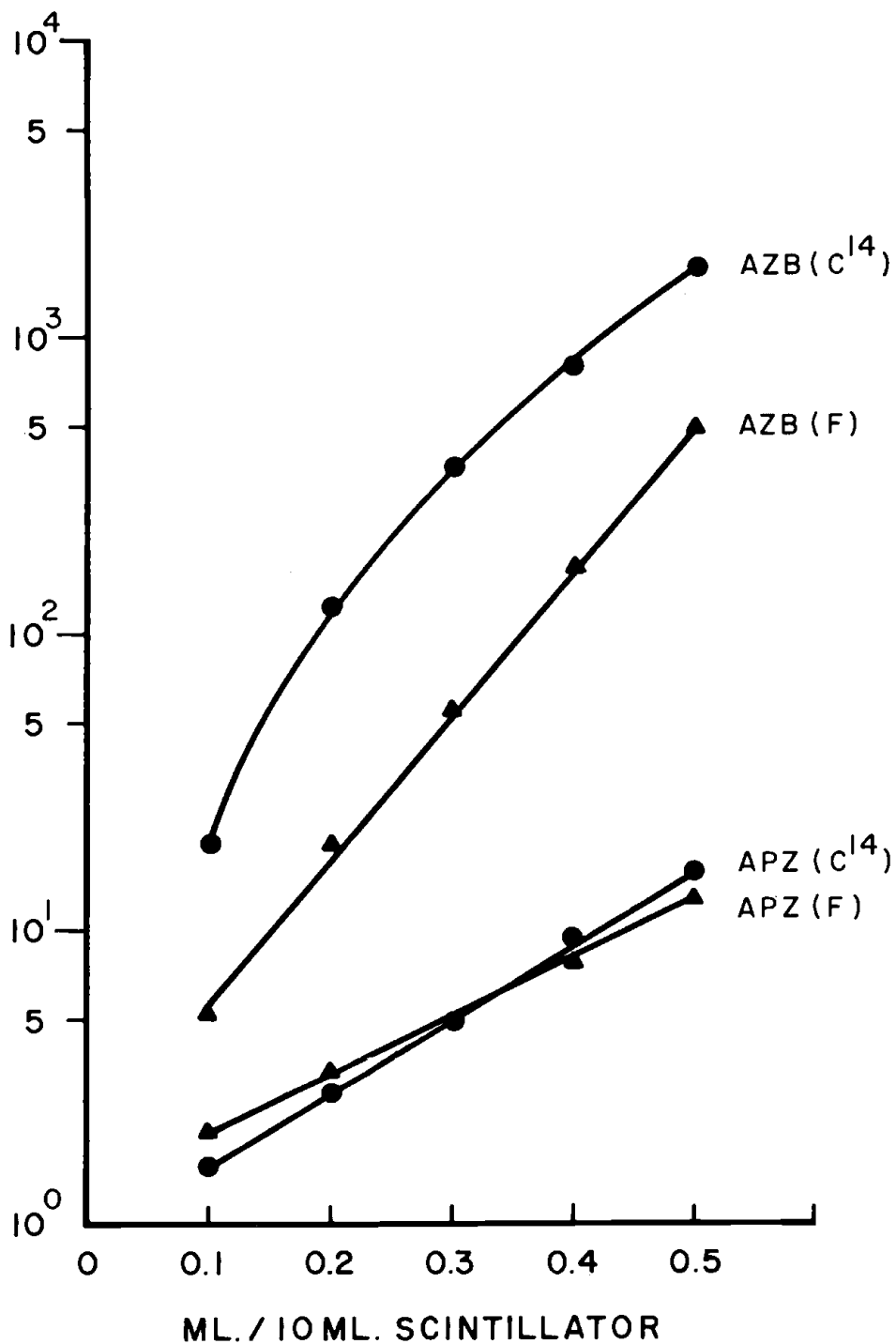


Fig. 5. See text. (F) and (C<sup>14</sup>) indicate data obtained using ultraviolet light and ionizing radiation, respectively, as sources of excitation.

## Summary

The results of this study indicate the following:

- 1) The internal standard method and the extrapolation method yield equally valid and accurate results in quenching correction in liquid scintillation counting under conditions of minimal quenching or dilute sample concentration.
- 2) The accuracy of the results is dependent upon the beta energy of the isotope.
- 3) In cases of severe quenching, the internal standard method tends to yield inaccurate but consistently high results; whereas, under identical experimental conditions, the extrapolation method may or may not apply depending upon the degree of severity of quenching involved.
- 4) At high sample concentrations of the quencher, the extrapolation method may not apply if a deviation from linearity of the semi-log plot of apparent specific activity vs. sample concentration is observed.
- 5) In order to obtain an accurate factor for quenching correction in the internal standard method, comparable or higher activity than sample activity should be used as internal standard.
- 6) For colored or transparent samples containing stronger quenchers, the background count is lower than that of liquid scintillator alone and tends to approach a limiting value.

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