Determination of the Liquid Scintillation Counting Efficiency of ^3H and/or ^{14}C Labelled Samples Independently of the Degree of Colour and/or Chemical Quenching

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(Z. Naturforsch. **32 c**, 143–149 [1977]; received December 29, 1976)

Liquid Scintillation Counting, Efficiency Determination, Chemical and/or Colour Quenching

A value R is obtained by the ratio of the summed, coincident pulses of both photomultipliers to the coincident pulses of one photomultiplier in the same selected range of the external standard puls height spectrum. This value R together with the external standard channel ratio (ESCR) show the extent of chemical and/or colour quenching in a field described by a set of calibration samples with increasing chemical or colour quenching (Fig. 3). The counting efficiency of samples with different and unknown proportion of chemical and/or colour quenching can be determined much more accurate than by any calibration approach using the ESCR only (Table VI). The method can be used for ^3H samples with a counting efficiency $\geqq 10\%$ and ^{14}C samples of $\geqq 5\%$, respectively. The new correction procedure is also applicable for ^3H and ^{14}C double-labelled compounds.

The count rate (cpm) indicated by a measuring instrument will always represent only a fraction of the actual decay rate of a radioactive sample (dpm). The cpm/dpm ratio is a measure of the counting efficiency (E). In liquid scintillation measurements this efficiency depends, under otherwise unchanged conditions, on the degree of quenching in the sample [1–4]. The term "quenching" describes disturbances during the energy transfer from the emitted negatron up to the photons detected by the photomultipliers. Consequently, establishing the dpm rate first requires that the quenching state or counting efficiency is determined [1–4]. The external standard channel ratio (ESCR) method is now the most widely used amongst the various alternative quench correction methods, mainly because of its suitability for automation. It is based on the fact that quenching causes a pulse height shift [5] in a Compton spectrum generated in the scintillator solution by external

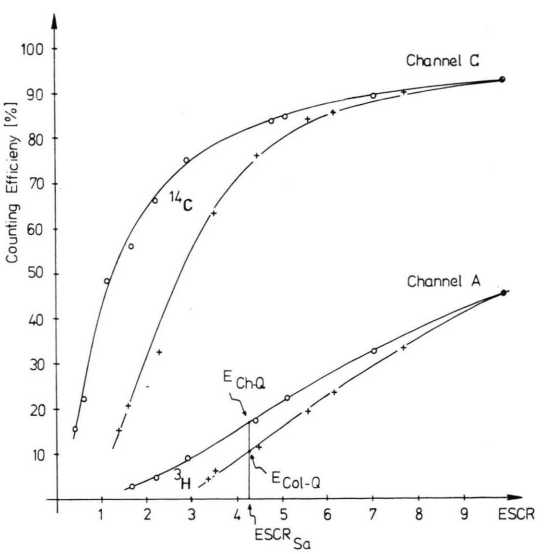

Fig. 1. Relationship between counting efficiency (E) and external standard channel ratio (ESCR) for ^3H and ^{14}C calibration samples with increasing degree of purely chemical quenching (○○○) or mainly colour quenching (+++). The curve representing tritium calibration samples illustrates that a sample with the $ESCR_{Sa}$ and unknown quenching will generally have a counting efficiency between $E_{CH\text{-}Q}$ (intersection between $ESCR_{Sa}$ and the curve defined by chemically quenched calibration samples) and $E_{COL\text{-}Q}$ (intersection between $ESCR_{Sa}$ and the curve defined by colour quenched calibration samples).

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Abbreviations: ES, external standard; ESCR, external standard channel ratio; ${}_x\mathrm{cpm}_y$, counts per minute of nuclide (or sample) x in measuring channel y; dpm, absolute decays per minute (decay rate); E, counting efficiency; CH-Q, chemical quench; COL-Q, colour quench; Sa, sample (containing nuclide x); DU, discriminator unit; PM, photomultiplier.

gamma irradiation [6]. Calibration samples with a known counting efficiency, whose ESCR is determined, provide quench correction functions ($E = f(\mathrm{ESCR})$) (*cf.* Fig. 1). Diagrams representing these functions allow the counting efficiency for unknown quenched samples to be read off once their ESCR has been established.

One major disadvantage of the ECSR method is the fact that the E/ESCR functions vary depending on the type of quenching (*cf.* Fig. 1). The reasons are the various quenching mechanisms. Chemical quenching produces disturbances in the energy transfer between solvent molecules and also between these molecules and the scintillator molecules where the energy is not ultimately given off in the form of photons. Colour quenching absorbs photons on their way from their point of origin to the photomultipliers.

When exclusively chemically quenched samples and others which are wholly or mainly colour quenched are plotted as curves in the E/ESCR diagram then the divergence between the curves increases with the degree of quenching [7, 8]. In practice, however, samples frequently combine both colour and chemical quenching in different degrees.

In order to overcome the practical problems of diverging E/ESCR functions it would be necessary to establish a separate quench correction factor for each type of sample. This, however, is not feasible with the majority of samples in which the quenching make-up is mostly highly complex and unpredictable.

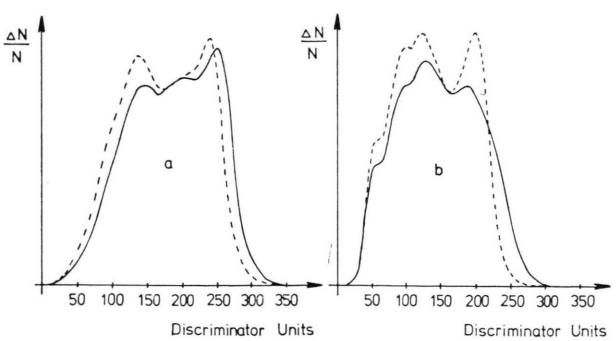

Fig. 2. Pulse height spectra of ^{137}Cs as the external standard, produced in scintillator samples with the same counting efficiency which have been chemically quenched (– – –) by adding carbon tetrachloride, and colour quenched (———) by adding piceine.
a. The spectra were obtained by summation of the count pulses of two coincident photomultipliers.
b. Pulse height spectra of samples as in 2 a, but with only one of the two coincident photomultipliers being evaluated.

Observations by the authors and by others [3, 8–11] have shown that chemically and colour quenched samples have different pulse height spectrum forms (*cf.* Fig. 2 a). Chemically quenched samples cover a smaller pulse height range than colour quenched samples. The wider range of colour quenched spectra is the result of the fact that the photon absorption varies with the path length (Lambert-Beer's Law). In counters arranged in a coincident configuration and equipped for pulse summation, the summation causes spectral "over-weighting" of the pulse which has to pass through the shorter distance to the photomultiplier. Compared with its coincident partner, this pulse produces a greater pulse height in the photomultiplier [3, 9, 11, 12].

The lesser pulse height analysis method utilises this difference in behaviour between two coincident pulses to correct chemically and/or colour quenched samples [8]. As only the smaller of two coincident pulses is passed on for pulse height analysis, the spectra of chemically and colour quenched samples are standardized. According to [8] the same principle applies to ES spectra so that one quench correction function can be used for weighting all samples. Apart from the fact that this method requires additional electronic hardware at the spectrometer, a more specific disadvantage is that the absence of pulse summation reduces the spectral resolution [3]. This would have a particularly unfavourable effect on the analysis of double-labelled samples (*e. g.* ^{3}H/^{14}C). We therefore made the attempt to utilize the spectrum difference between chemically and colour quenched samples in a different way for determining the counting efficiency irrespective of the type of quench involved.

Results and Discussion

If the spectrum for a quenched sample obtained with only one photomultiplier through the external standard and under coincidence conditions is compared with the spectrum of both photomultipliers, then differences will be found (Figs 2 a, b). If a channel is formed in a specially selected low-energy range then the following ratio can be calculated for a sample:

$$R = \frac{{}_{ES}^{1}\mathrm{I} - {}_{Sa}^{1}\mathrm{I}}{{}_{ES}^{2}\mathrm{I} - {}_{Sa}^{2}\mathrm{I}} \qquad (1)$$

where ${}_{ES}^{1}\mathrm{I}$ represents the number of count pulses in the above-mentioned channel which are supplied

by one photomultiplier, and $_{ES}^{2}I$ the number of count pulses in the same channel for the spectrum obtained with two photomultipliers. In order to obtain the net ES rate, $_{ES}^{1}I$ and $_{ES}^{2}I$ must be reduced by the samples rate $_{Sa}^{1}I$ and $_{Sa}^{2}I$. If R is calculated for different samples then it will be found that R remains virtually constant even when the chemical quench in the samples increases. In samples with increasing colour quench, however, R becomes bigger (*cf.* Fig. 3). At a given ESCR, therefore, R will

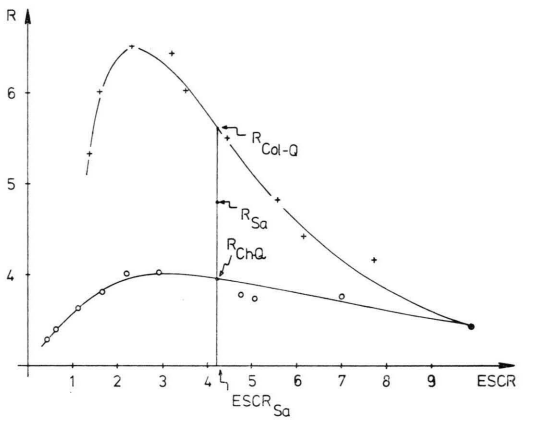

Fig. 3. The diagram shows R (*cf.* equation 1) as a function of the external standard channel ratio (ESCR) for chemically quenched (○○○) or mainly colour quenched (+++) calibration samples (see also calculation example).

provide an indication of the ratio between colour and chemical quenching. A curve representing R as a function of the ECSR for a series of samples with increasing colour quench will therefore be different from the curve for a series of samples with increasing chemical quenching. Similarly, in an R/ESCR diagram the two curves will be divergent (Fig. 3), as will the curves in the E/ESCR diagram for both series of samples.

By linear plotting of the R/ESCR characteristic in the E/ESCR diagrams it is possible to determine the counting efficiency of samples with an unknown degree and ratio of colour and chemical quenching (Table I).

Table I shows the counting efficiency, the ESCR and the value of R for seven ^{3}H and ten ^{14}C calibration samples (quenching steps) with an increase in concentration of carbon tetrachloride. For the ^{14}C samples the counting efficiency is listed for three different channels. Channels A and B are suitable for ^{14}C measurements in $^{3}H/^{14}C$ labelled samples. Table II shows the corresponding figures for a batch of increasingly colour quenched samples. The E/ESCR characteristics for both batches of calibration samples (sample preparations see experimental part) are illustrated in Fig. 1. The phenomenon of divergence between the curves for colour and chemically quenched samples has been described as early as 1960 by Baillie[2]. It can instantly be recognized that determining the counting efficiency of wholly or predominantly colour quenched samples by means of an E/ESCR function which is based on a set of purely chemically quenched samples would result in major errors and vice versa. The degree of these errors ($\% \Delta E_{CH\text{-}Q}$ and $\% \Delta E_{COL\text{-}Q}$) is shown in Table III – V. In double-labelled samples the same circumstances would produce even much greater errors.

Table III lists the counting efficiencies of various samples obtained by mixing different samples as described in the experimental section. The column headings denote the following:

Table I. Counting efficiency of ^{3}H and ^{14}C labelled calibration samples in channels A, B and C, plus corresponding ESCR and R values for chemical quenching by carbon tetrachloride.

Quenching step	µl CCl_4 per calibration sample	E (^{3}H)	E (^{14}C)			ESCR	R
		A	A	B	C		
1	0	45.50	30.01	63.09	93.05	9.887	3.441
2	20	32.48	46.04	44.00	89.85	7.039	3.762
3	45	22.18	59.74	25.65	85.18	5.098	3.739
4	60	17.31	60.48	23.80	84.09	4.772	3.773
5	100	9.00	72.02	3.15	75.11	2.921	4.021
6	150	4.89	66.01	0.44	66.44	2.202	3.988
7	200	2.74	56.07	—	56.11	1.673	3.810
8	300	—	38.76	—	38.76	1.131	3.636
9	400	—	22.00	—	22.02	0.642	3.396
10	500	—	15.70	—	15.72	0.433	3.288

Table II. Counting efficiency of ^{3}H and ^{14}C labelled calibration samples in channels A, B and C plus corresponding ESCR and R values for colour quenching by piceine.

Quenching step	µl piceine solution per calibration sample	E (^{3}H)	E (^{14}C)			ESCR	R
		A	A	B	C		
1	0	45.50	30.01	63.09	93.05	9.887	3.441
2	20	33.52	43.44	46.88	90.14	7.731	4.173
3	45	23.54	53.82	32.01	85.63	6.187	4.427
4	60	19.23	59.16	25.20	84.17	5.590	4.817
5	100	11.33	64.82	11.30	75.97	4.484	5.507
6	150	6.06	59.92	3.41	63.27	3.515	6.033
7	200	4.31	—	—	—	3.192	6.483
8	300	—	32.01	—	32.60	2.308	6.519
9	400	—	20.17	—	20.49	1.604	6.000
10	500	—	15.16	—	15.39	1.378	5.333

Table III. Comparison of the percentage deviation of counting efficiencies (% ΔE) determined by the method described, and the deviation of counting eficiencies (% $\Delta E_{CH\text{-}Q}$ and % $\Delta E_{COL\text{-}Q}$) determined on the basis of E/ESCR functions obtained with purely chemically quenched or with colour quenched calibration samples. Quenching of the ^{3}H and ^{14}C samples was achieved by adding different proportions of carbon tetrachloride and piceine (*cf.* sample preparation).

Sample	Nucleide	Meas. channel	ESCR	R	E_{theor}	E	% ΔE	% $\Delta E_{CH\text{-}Q}$	% $\Delta E_{COL\text{-}Q}$
A1	^{3}H	A	4.98	4.17	15.6	15.4	− 1.2	+10.9	−36.5
	^{14}C	C			81.6	81.6	+ 0.1	+ 2.9	− 9.4
		B	4.96	4.18	19.1	19.2	+ 0.3	+12.4	−35.1
		A			63.2	62.8	− 0.6	+ 0.1	− 2.7
A2	^{3}H	A	3.20	4.45	7.1	7.1	+ 0.1	+19.7	−87.3
	^{14}C	C			70.2	69.0	− 1.7	+ 5.7	−33.3
		B	3.22	4.45	3.1	3.7	+17.5	+39.5	−78.3
		A			67.6	66.3	− 1.9	+ 5.0	−32.0
A3	^{14}C	C	2.13	4.45	52.6	53.6	+ 2.0	+17.2	−57.0
B1	^{3}H	A	6.03	4.31	18.8	18.0	− 4.3	+21.8	−16.5
	^{14}C	C			84.1	83.6	− 0.6	+ 3.3	− 2.5
		B	6.02	4.32	26.7	26.8	+ 0.6	+18.8	− 8.2
		A			57.6	57.7	+ 0.2	+ 2.5	− 0.8
B2	^{3}H	A	4.44	4.96	9.5	10.0	+ 5.1	+52.6	−24.0
	^{14}C	C			73.7	72.6	− 1.5	+12.8	−10.2
		B	4.40	4.94	9.2	9.1	− 1.7	+52.9	−34.9
		A			64.9	65.6	+ 1.0	+ 8.6	− 3.6
B3	^{14}C	C	3.13	5.73	58.1	52.3	−10.0	+25.4	−24.0

Table IV. Comparison of percentage deviation as in Table III, for 10 ml ^{3}H samples measured in channel A.

Sample/ quenching substance	ESCR	R	E_{theor}	E	% ΔE	% $\Delta E_{CH\text{-}Q}$	% $\Delta E_{COL\text{-}Q}$
Urine 500 µl	4.18	4.01	15.7	15.5	−0.9	+ 0.6	−51.4
Spinach extract 5 µl	4.77	3.77	19.7	19.8	+0.5	+ 4.4	−36.2
Spinach extract 50 µl	3.33	4.47	11.4	10.7	−5.7	+ 8.1	−61.3
Blood 20 µl	4.10	5.39	9.7	8.7	−9.6	+ 53.2	−27.5
Blood 40 µl	2.92	5.86	4.1	4.4	+7.3	+116.7	−43.3
Sudan III 0.1 mg	4.65	5.48	11.2	11.6	+3.3	+ 78.1	− 3.3
Sudan III 0.2 mg	3.19	6.51	3.5	3.6	+2.6	+233.3	− 2.6
Azobenzene 0.5 mg	7.22	4.58	28.4	27.4	−3.5	+ 15.4	+ 6.5
Azobenzene 1.0 mg	5.36	5.27	17.3	15.8	−8.4	+ 32.3	+ 2.2
Azobenzene 2.0 mg	3.70	5.85	7.3	7.1	−3.6	+ 82.6	−13.1
Benzanthracene 0.22 mg	6.48	4.26	23.3	23.9	+2.4	+ 14.9	− 5.7
Benzanthracene 1.00 mg	4.26	4.80	11.4	11.9	+3.9	+ 35.7	−30.8

Table V. Comparison of percentage deviation as in Table III, for 10 ml ^{14}C samples.

Sample	Channel	ESCR	R	E_{theor}	E	$\% \Delta E$	$\% \Delta E_{CH\text{-}Q}$	$\% \Delta E_{COL\text{-}Q}$
Urine 500 μl	C	4.15	3.97	81.7	81.7	+ 0.1	+ 0.1	−16.5
Spinach extract 5 μl	C	4.81	4.09	85.9	83.6	− 2.7	+ 2.2	− 8.0
Spinach extract 50 μl	C	3.32	4.34	77.9	75.2	− 3.4	+ 0.4	−22.0
Blood 40 μl	C	4.02	5.48	73.2	69.3	− 5.3	+ 12.0	− 9.6
Blood 20 μl	C	2.78	6.10	52.8	49.4	− 6.6	+ 37.4	−19.4
Sudan III 0.1 mg	C	4.63	5.69	74.9	76.2	+ 1.7	+ 11.7	− 2.9
Sudan III 0.2 mg	C	3.23	6.66	50.1	57.1	+14.0	+ 54.0	−17.3
Azobenzene 0.5 mg	C	6.92	4.50	88.5	88.0	− 0.6	+ 0.3	− 0.3
	B			38.3	38.8	+ 1.5	+ 12.0	+ 5.4
	A			50.5	50.7	+ 0.4	− 3.9	− 1.2
Azobenzene 1.0 mg	C	5.44	5.17	83.4	81.6	− 2.1	+ 3.2	− 0.9
	B			22.4	21.5	− 3.7	+ 34.2	+ 5.1
	A			61.1	59.7	− 2.4	− 6.4	− 3.3
Azobenzene 2.0 mg	C	3.66	5.95	68.0	71.0	+ 4.5	+ 16.9	− 1.3
	B			3.9	5.0	+29.2	+151.9	+20.8
	A			64.2	60.7	− 5.4	+ 3.3	− 6.0
Benzanthracene 0.22 mg	C	6.21	4.40	85.9	86.1	+ 0.2	+ 5.6	− 0.7
Benzanthracene 1.00 mg	C	4.18	4.97	76.0	74.8	− 1.5	+ 7.7	− 8.9

E_{theor} was calculated on the basis of the known radioactivity of the [^3H] and [^{14}C] *n*-hexadecane standards added, E is the counting efficiency determined by the method described in this paper, $\% \Delta E$ denotes the deviation from E_{theor}, $\% \Delta E_{CH\text{-}Q}$ and $\% \Delta E_{COL\text{-}Q}$ indicate the errors introduced by the use of E/ESCR functions based on purely chemically or colour quenched samples.

In addition to measurements in the integral channels A for ^3H and C for ^{14}C, ^{14}C measurements were carried out in channels B and A. The lower threshold of the integral ^{14}C channel in channel B was raised to a level at which the least quenched ^3H sample no longer produced any count pulses. Channel A registers, in addition to all tritium counts, the low-energy part of the ^{14}C spectrum. The errors $\% \Delta E$ observed with the method used were without exception substantially smaller than the errors $\% \Delta E_{CH\text{-}Q}$ and $\% \Delta E_{COL\text{-}Q}$ respectively. The method reaches its limits at efficiencies of $<5.5\%$ for ^{14}C and $<10\%$ for ^3H in integral measuring channels, and $<10\%$ for ^{14}C in channel B.

After having obtained these results, a number of samples from practical day-to-day applications were checked (Tables IV and V). These can be divided into three groups (*cf.* Sample Preparation):

a) mainly chemical quenched (urine, spinach);
b) mainly colour quenched (blood, Sudan III dye, azobenzene), and
c) mixed samples (benzanthracene).

As expected, the error $\% \Delta E_{COL\text{-}Q}$ was greatest in the first group, whereas E/ESCR functions obtained by chemically quenched standards produced corrections which were still within acceptable limits. The converse is true for the second group. In the third group, the E/ESCR functions fail both with chemically quenched and colour quenched samples. The method described produces satisfactory results for all three groups. The limitations outlined in the discussion of Table III again apply in this instance. With the ^{14}C azobenzene samples additional measurements were carried out in channels B and A corresponding to double-labelled samples. The relatively large error in channel B observed with the 200 μl sample is very likely due to the fact that the lower threshold in B was set too high for this quench. The ^3H labelled azobenzene samples showed special peculiarities: the positive sign for $\% \Delta E_{COL\text{-}Q}$ indicates that the E_{theor} values of these samples were not between $E_{CH\text{-}Q}$ and $E_{COL\text{-}Q}$ but even below the E/ESCR characteristic obtained with piceine calibration samples (*cf.* Fig. 1). Nevertheless, the value of R for these samples still produced adequate results and allows the counting efficiency of these samples to be corrected. The situation is similar as regards the counting efficiency of ^{14}C samples measured in channel B. This underlines two advantages of this correction method: a) the R/ESCR ratio allows any E/ESCR function to be corrected irrespective of the measuring channel and nuclide for which these functions have been worked out. This is par-

ticularly useful for correcting double-labelled samples. b) Although it is expedient to establish the required E/ESCR and R/ESCR functions on the basis of purely chemically quenched or mainly colour quenched samples, the choice of colour quencher is obviously not critical since the linear representation of R values remains correct even outside the R/ESCR field (*cf.* Fig. 3). Table VI shows the mean value of the absolute errors of all samples listed in Tables III – V in all channels. This comparison illustrates the potentials and capabilities of the method described in this paper.

Table VI. Comparison of mean values of absolute errors of all samples from Tables III–V (cf. legend Table III).

Nuclide/ Channel	Number of samples	$\% \Delta E$	$\% \Delta E_{\text{CH-Q}}$	$\% \Delta E_{\text{COL-Q}}$
^{3}H/A	16	3.9	48.8	28.0
^{14}C/C	18	3.3	12.2	13.6
^{14}C/B	7	7.8	46.0	26.8
^{14}C/A	7	1.7	4.3	7.1

Experimental Section

a) Description of equipment

The liquid scintillation counter used was a laboratory model BF 5000 made by Comp. Lab. Prof. Berthold (D-7547 Wildbad) with three independent measuring channels A, B and C. The ESCR was determined with ^{137}Cs, two of the above channels (channel D and E) being changed over automatically to the following relationship:

$$\text{ESCR} = \frac{\text{ES}^{\text{cpm}}\,\text{D} - \text{Sa}^{\text{cpm}}\,\text{D}}{\text{ES}^{\text{cpm}}\,\text{E} - \text{Sa}^{\text{cpm}}\,\text{E}} \,. \qquad (2)$$

The value of R (*cf.* equation 1) is established (*cf.* Fig. 4) by measuring the coincident added-pulse signals of the external standard nuclide of both photomultipliers (switch S closed) and by measuring the coincident signals from one photomultiplier only (switch S open). In order to determine the net count rate of the external standard an similar measurement is carried out on the non-irradiated sample.

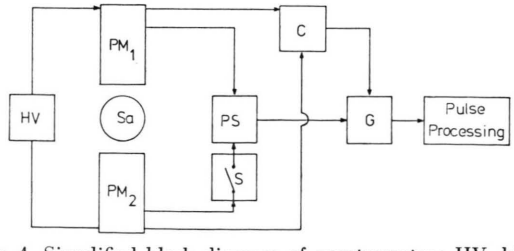

Fig. 4. Simplified block diagram of spectrometer: HV, high-voltage supply; PM, photomultiplier; Sa, sample; PS, pulse summation; C, coincidence stage; G, gate which only passes on coincident pulses to the pulse height analyzer and recorder; S, switch for isolating one PM from the PS stage.

The measuring process is divided into three phases:

1) Determining the value of R, 2) determining the ESCR, and 3) the sample measurement proper.

b) Sample preparation

To approx. 1.0×10^5 dpm [^{3}H]*n*-hexadecane and approx. 0.5×10^5 dpm [^{14}C]*n*-hexadecane (standards supplied by Comp. Amersham Buchler, D-3301 Braunschweig) were added 10 ml toluene scintillator (5.0 g of 2,5-diphenyloxazol and 0.5 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene per litre toluene). For chemical quenching, increasing quantities of carbon tetrachloride were added to a series of calibration samples (*cf.* Table I). Similarly, to produce colour quenching, increasing quantities of a solution consisting of 1.0 g piceine per litre toluene were added to a second series of samples (*cf.* Table II).

The method was checked by means of samples which, in addition to defined quantities of [^{3}H] and/or [^{14}C]*n*-hexadecane standards, also contained different quantities of carbon tetrachloride and piceine, plus the quenching substances listed in Tables IV and V. In Table III, A and B denote samples containing different quantitative proportions of carbon tetrachloride and piceine. Figs 1 – 3 denote increasing quantities of the same proportion of carbon tetrachloride and piceine.

Urine samples: 500 µl were dissolved in 10 ml 2-phenylethylamine:methanol:toluene = 18:25:57 (per litre of this mixture 5.0 g of 2,5-diphenyloxazol and 0.5 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene).

Spinach extract: the aliquots listed in Tables IV and V of an aqueous chlorophyll suspension of spinach leaves (0.237 mg chlorophyll/ml) were dissolved in 10 ml of the scintillator described for the urine samples.

Blood: 2 ml of complete blood and 8 ml of soluene 350 (Comp. Packard Instruments, D-6000 Frankfurt) were stirred for 0.5 h at approx. 55 °C. After the solution was allowed to cool, the aliquots corresponding to the volumes of blood listed in Tables IV and V were dissolved in 10 ml of toluene scintillator (see above).

Sudan III: Stock solution: 1 mg of Sudan III in 1 ml of toluene. Aliquots corresponding to the volumes listed in Table IV and V were then dissolved in 10 ml of toluene scintillator (see above).

Azobenzene: Stock solution: 1 g azobenzene in 100 ml of toluene. Of this, aliquots corresponding to the volumes listed in Tables IV and V were dissolved in 10 ml of toluene scintillator.

Benzanthracene: the weights listed in Table IV and V were dissolved directly in 10 ml of toluene scintillator.

c) Calculation example for the correction method. Any given sample is described by the following parameters: a) ESCR and b) R value, and in the case of check samples c) $E_{\text{theor}} = \text{cpm/dpm}$. The unknown is the counting efficiency E which, under optimum conditions, would be identical with E_{theor}. This is obtained by linear representation of the R/ESCR function in the E/ESCR function as follows:

The vertical line above the ESCR of the sample intersects with the E/ESCR functions at $E_{\text{CH-Q}}$ and $E_{\text{COL-Q}}$ (*cf.* Fig. 1), and with the R/ESCR functions at $R_{\text{CH-Q}}$ and $R_{\text{COL-Q}}$ (*cf.* Fig. 3) respectively. Using the R value of the sample R_{Sa}, the following equation can be established:

$$(R_{\text{CH-Q}} - R_{\text{Sa}}) : (R_{\text{CH-Q}} - R_{\text{COL-Q}}) = (E_{\text{CH-Q}} - E) : (E_{\text{CH-Q}} - E_{\text{COL-Q}}) \quad (3)$$

which is solved to find E as follows:

$$E = E_{\text{CH-Q}} - \frac{(E_{\text{CH-Q}} - E_{\text{COL-Q}})}{(R_{\text{CH-Q}} - R_{\text{COL-Q}})} \times (R_{\text{CH-Q}} - R_{\text{Sa}}). \quad (4)$$

Tables III – V list the percentage deviation $\% \Delta E$ relative to E_{theor} as calculated in accordance with Eqn (5):

$$\% \Delta E = \frac{(E - E_{\text{theor}})}{E_{\text{theor}}} 100. \quad (5)$$

$\% \Delta E_{\text{CH-Q}}$ and $\% \Delta E_{\text{COL-Q}}$ are calculated similarly.

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