facturing methods were designed. And now we are once again in a period of declining interest. It is to be hoped that no new threat will be needed to generate a renewed wave of investigations. Let us hope that the intellectual challenge alone will bring support for activities that will lead to increased understanding of the botulinal toxins (*30*).

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Self-Absorption Correction for Carbon-14

A new treatment yields a correction factor that is linearly related to the thickness of the sample.

Richard W. Hendler

Carbon-14 is undoubtedly the most widely used isotope in biochemical research. However, because of its weak emission, beta particles of maximum energy 0.156 Mev, there is an appreciable absorption and scattering of radioactivity within the sample itself, as a function of the weight of the sample. It is of the utmost importance to be able to correct accurately for the loss of observable radioactivity due to self-absorption (*1*). In the past the problem has been dealt with by (i) counting at "infinite thinness"; (ii) counting at constant thickness; (iii) counting at "infinite thickness"; (iv) correcting by use of an empirically determined curve; (v) or correcting by use of a theoretical equation with an empirically determined constant.

The thinner the sample, the less the self-absorption. Therefore, if all samples are counted at extremely low weights (infinite thinness), the correction will be negligible. This requires always having material of sufficiently high specific radioactivity to permit accurate determinations on very small quantities. The criterion is frequently difficult to meet in biochemical work.

If the samples counted are always of the same thickness, the correction factor will always be the same, and the results will all be comparable. It is very tedious to weigh all samples to exactly the same weight and this method is rarely used.

The observable count rate of a material of given specific activity will increase as the thickness of the sample is increased. The lowest layer contributes less radioactivity to the total observed, as more material is added, since its radiation is progressively absorbed. There is a weight of material over a given area which will be sufficient to absorb virtually all of the radiation from this lowest layer. Adding more radioactive material after this does not increase the total observed radioactivity, since the effective thickness of radioactive material contributing to the total observable radiation is essentially not altered. This is known as infinite or saturation thickness, and the maximum count rate observed from a sample whose thickness is greater than the saturation thickness is directly proportional to the specific activity of the material. This technique is quite reliable but requires relatively large amounts of material and therefore is not always convenient in biological systems.

The most general technique involves the use of a correction factor so that the

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radioactivity determined at any weight can be expressed in terms of equivalent radioactivity that would be observed under some previously selected reference condition. The reference condition can be either "infinite thinness," "infinite thickness," or a fixed finite weight. A curve for correction factor versus weight (in milligrams) of sample can be obtained from a series of planchets of fixed area, containing samples of different weights of a material of a particular specific activity. The empirically determined correction factor can then be read from the curve for a sample of any weight and used to correct to the standard condition. The accuracy of the curve increases with the number of experimental points determined, and it seems necessary to plot at least 25 points to determine accurately a fair range of the curve. The correction factor for samples of low weight is very difficult to determine because of the increase in the percentage uncertainty of a weight as the weight is decreased to zero, difficulties of obtaining smooth distribution of material, and various other theoretical considerations (*2*).

An accurate theoretical equation would solve all of the difficulties, since it would enable one to know the correction factor at any weight through simply solving or plotting the equation.

An equation intended to describe the absorption phenomenon was derived at about the same time by Libby (*3*) and by Henriques *et al.* (*4*). The equation is a general one, with a constant which can be determined with a few samples. The derivation of this equation follows.

If it is assumed that each layer (dm) of material will absorb the same percentage (α) of the impinging radiation, then

$$-\frac{dI}{dm} = \alpha I$$

$$\ln I = -\alpha m + k'$$

$$I = k'' e^{-am} \qquad (1)$$

At $m = 0$, $k'' = I_0$,

$$\therefore I = I_0 e^{-am} \qquad (2)$$

where I is the observed count rate in counts per minute, I_0 is the count rate at zero absorption, m is the weight of the sample over a fixed area, and α is the fraction of radiation lost when the radiation passes through a layer of material dm. Equation 2 is the typical Beer's Law expression. Now if the material itself is radioactive, then for a sample of constant area and geometry an increment of material will have an initial activity $dI_0 = \sigma dm$, where σ is the observed specific activity in counts per minute per milligram at infinite thinness and dm is the weight of the layer increment. Thus:

$$\int_0^I dI = \sigma \int_0^m e^{-am} dm \qquad (3)$$

Integrating from the top of the sample downwards,

$$I = \frac{\sigma(e^{-am} - 1)}{-\alpha} = \frac{\sigma}{\alpha}(1 - e^{-am}) \qquad (4)$$

where σ is the specific activity of the material at zero absorption in counts per minute per milligram. At $m = \infty$,

$$I_\infty = \frac{\sigma}{\alpha}(1 - 0) = \frac{\sigma}{\alpha}$$

where I_∞ is the maximum count rate observed at infinite thickness (m_∞).

$$\therefore I = I_\infty(1 - e^{am})$$

or

$$I = I_\infty(1 - 10^{-a^{1}m.}) \qquad (5)$$

This is the standard equation for exponential absorption of radioactivity. It can be alternatively derived by stating that each increment dm increases the radioactivity by virtue of its specific activity σ and decreases it by virtue of blocking a constant fraction α of the impinging radiation I.

$$\therefore \frac{dI}{dm} = \sigma - \alpha I \qquad (6)$$

$$\frac{dI}{dm} + \alpha I = \sigma \qquad (7)$$

This is a standard linear differential equation [see Reddick (*5*)], the solution and evaluation of the constants of which lead again to Eq. 5. Equation 5 has been fitted to series of data so that it has satisfactorily described the absorption phenomenon over workable ranges of the curve (*6*). In practice it has been found that corrections based on this curve for samples of widely different weights can introduce serious errors. Although Libby has recently refined the equation by introducing specific constants for geometry and back scattering (*7*), the basic form of the equation has not been altered. In his paper, however, Libby

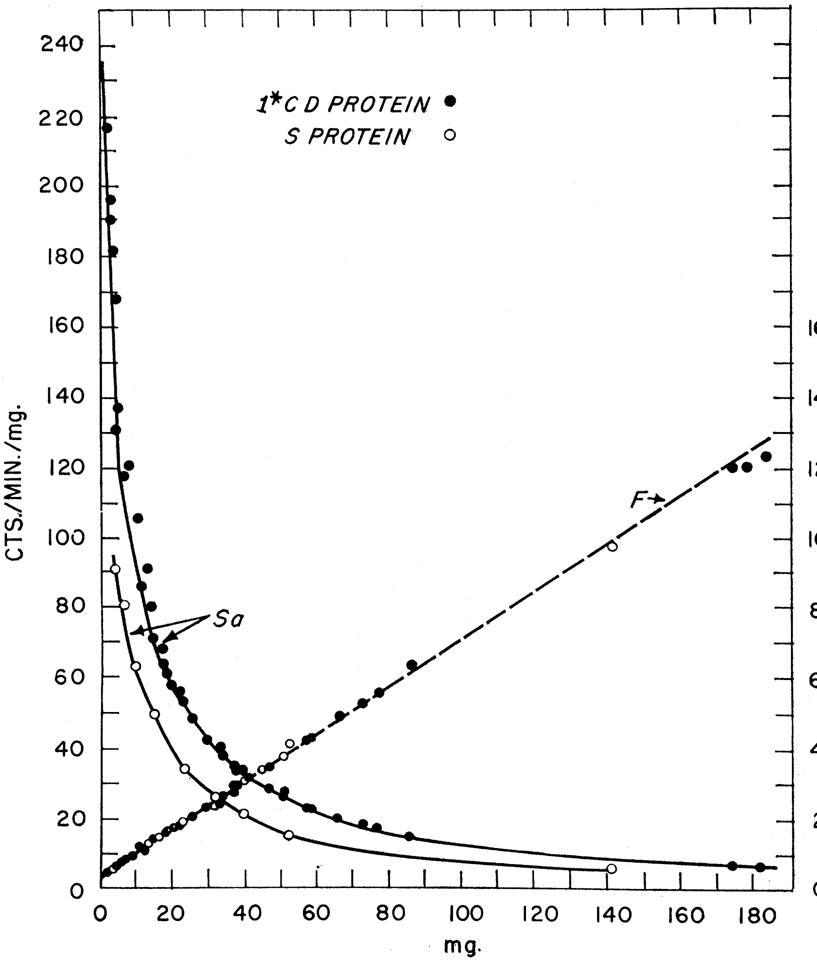

Fig. 1. A plot of observed specific activity and absorption correction factor *F* versus weight of sample for counting arrangement No. 1.

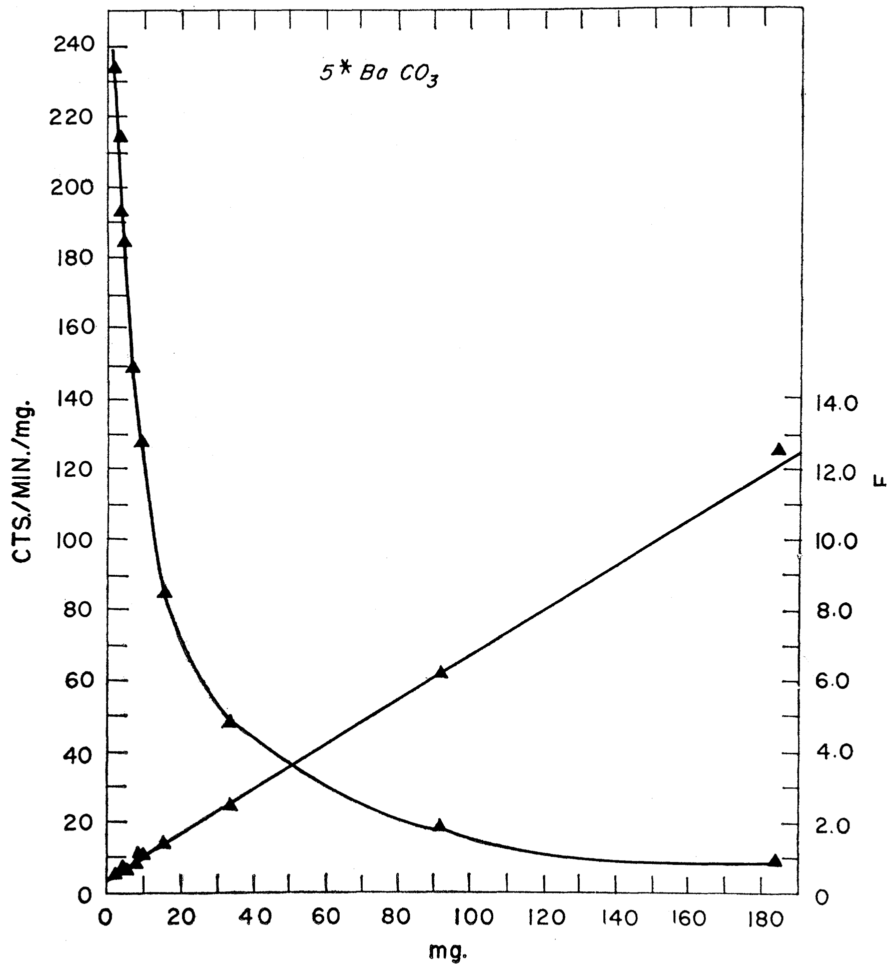

Fig. 2. A plot of observed specific activity (centimeters per minute per milligram) and absorption correction factor F (straight line) versus weight of sample (per milligram) for counting arrangement No. 5.

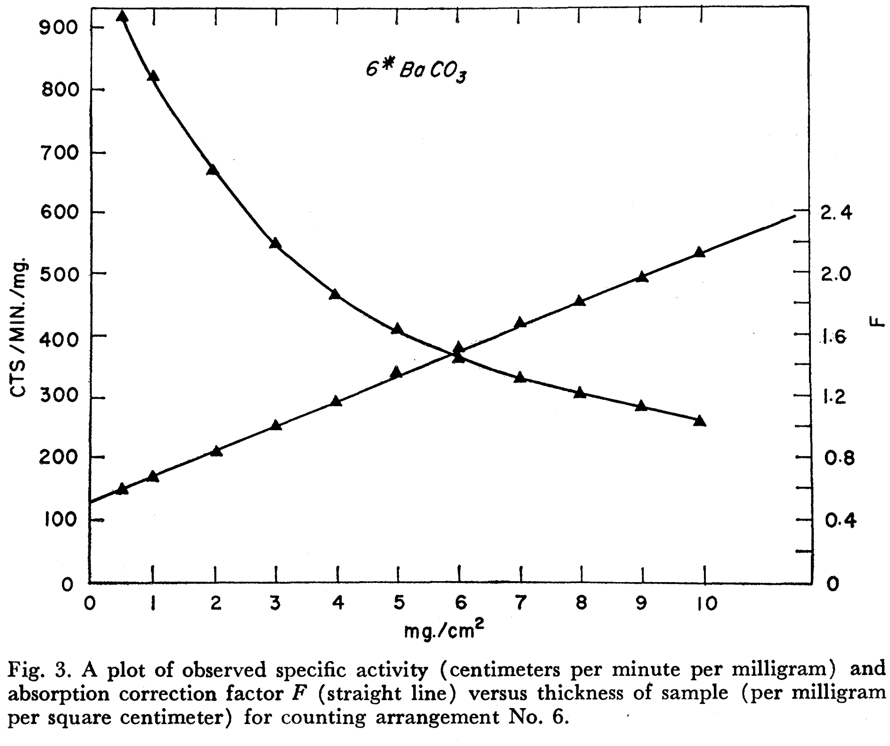

Fig. 3. A plot of observed specific activity (centimeters per minute per milligram) and absorption correction factor F (straight line) versus thickness of sample (per milligram per square centimeter) for counting arrangement No. 6.

stressed that true exponential absorption is not normally observed with the ordinary end-window type counter (7).

In practice it has been found in this laboratory that the apparent absorption coefficient α, instead of being constant, appears to decrease as a function of the weight of the sample. Furthermore, the interesting relation has been found that the apparent specific activity at any weight is inversely proportional to the weight of the sample. The decrease in apparent specific radioactivity appears to follow a hyperbolic, rather than a negative exponential, curve. If it is desired to correct the apparent specific activity at any weight to the specific activity at a reference weight, the factor F is used, having a value as follows:

$$F = \frac{S_{\text{ref}}}{S} = \frac{R}{S} = \frac{R}{I}m \qquad (8)$$

where S is the observed specific activity in counts per minute per milligram and S_{ref} equals R, which equals the specific activity at a reference weight. It is found that this factor, when plotted against the weight of the sample over a fixed area, gives a straight line from infinite thinness to a weight of several times saturation thickness. It can be seen that the slope of this line is equal to R/I_∞, and that the intercept b on the y-axis is equal to R/σ.

$$F = \frac{R}{I}m = \text{slope} \times m + b$$

$$\frac{R}{I} = \text{slope} + \frac{b}{m}$$

at $m = \infty$, the slope is R/I_∞.

$$\therefore F = \frac{R}{I_\infty}m + b \qquad (9)$$

Experimental Details

The following counting arrangements were used.

1) Nuclear Chicago scaling unit model 162. Nuclear Chicago model D47 gas-flow counter with an ultrathin "micromil" window. The tube was continually flushed with Q gas.

2) Nuclear Chicago scaling unit model 162. Tracerlab end-window counting tube TGC-2/1B84 with a window thickness of 1.9 mg/cm.

3) Same as in arrangement No. 2, with a layer of aluminum foil in front of the tube window.

4) Same as in arrangement No. 2, with the sample 0.8 cm further from the counting tube. The purpose of this ar-

Table 1. Robinson flow gas counter; $BaCO_3$; R at 10 mg; S, 118.

Thickness (mg/cm^2)	Wt. (mg)	Observed specific activity	Specific activity corrected to 10 mg with F		Error (%)	
			From hyperbolic treatment	From exponential treatment	Hyperbolic treatment	Exponential treatment
1.27	1.9	234	119	159	+1	+35
2.33	3.6	215	127	164	+8	+39
2.98	4.4	185	118	148	0	+25
5.97	9.2	128	120	129	+2	+ 9
22.5	33.4	48	119	103	+1	−13
59.3	91.7	19	118	92	0	−22
24.7	3.8	194	116	149	−2	+26
4.55	7.0	149	120	136	+2	+15
74.0	184.6	9.4	114	86	−3	−27
10.1	15.5	85	115	111	−4	− 6

rangement was to evaluate the possibility that the change in the distance between the surface of the sample and the counting tube with increasing weight of sample was a major factor in the linearity of F versus m. In counting arrangement No. 2 the planchet surface was about 4.0 mm from the counting-tube window. In counting arrangement 4, this distance was about 12 mm. The surface of the sample approaches the tube window 0.1 mm per 10 milligrams of protein. If the geometry change from 4.0 to 3.9 to 3.8, and so on, were responsible for the linearity of F versus m, then you would not expect the change from 12.0 to 11.9 to 11.8, and so on, to give a linear relationship. Since linearity was not apparently altered by this change, it appears that the relationship of F to m is not greatly dependent on this factor.

5) Nuclear Chicago scaling unit model 162. Robinson windowless gas-flow counter (*8*) flushed with 90-percent argon and 10-percent CO_2.

6) Potter Instrument Company scaling unit model 341A driven by a Radiation Counter Laboratories (RCL) nucleometer linear amplifier mark 15, model 1. Mark 12, model 1 (RCL) windowless flow gas tube flushed with methane gas and operated as a proportional counter.

The area of sample for counting arrangements Nos. 1 through 5 was 1.54 cm^2. The area of sample for counting arrangement No. 6 was 11.5 cm^2. In counting arrangements Nos. 1 through 5, the plane of the sample was perpendicular to the anode wire of the counter and was opposite the tip of the wire. In counting arrangement No. 6, the plane of the sample was parallel to the anode wire and exposed to the side of the wire.

Radioactive material counted consisted of (i) CD protein: cell debris proteins of hen oviduct (*9*); (ii) S protein: supernatant proteins of hen oviduct (*9*); (iii) $BaC^{14}O_3$; (iv) phenylalanine-3-C^{14}.

The technique for determining self-absorption correction factors is as follows. (i) Prepare a series of planchets (ten are sufficient) containing different known quantities of the same radioactive material; (ii) determine the count rate per milligram (specific activity) of each planchet; (iii) the factor (F) at any weight is equal to the ratio of the specific activity at a reference weight (R) to the observed specific activity; (iv) F times the observed specific activity equals the specific activity at reference weight, and F times the total observed activity equals the total activity observed at the reference weight.

A plot of F versus m was linear for all cases examined in this work. Because space is limited, the data for only a few of the different counting situations are presented here (Figs. 1–3).

One could strive to weigh out an exact weight of material for the desired reference weight. It has been found more convenient and more accurate (since many experimental points are used) to calculate the reference specific activity. This can be done by means of the following formula for several combinations of the data:

$$R = \frac{1 - G_{1,2}}{\frac{1}{S_1} - \frac{G_{1,2}}{S_2}} \qquad (10)$$

where R is reference specific activity; $G_{1,2}$ is $(m_1 - m_{\text{ref}})/(m_2 - m_{\text{ref}})$; S_1 is the observed specific activity of m_1 material; S_2 is the observed specific activity of m_2 material; and m_{ref} is reference weight in milligrams.

The factor F can be used to correct observed specific activity at any weight

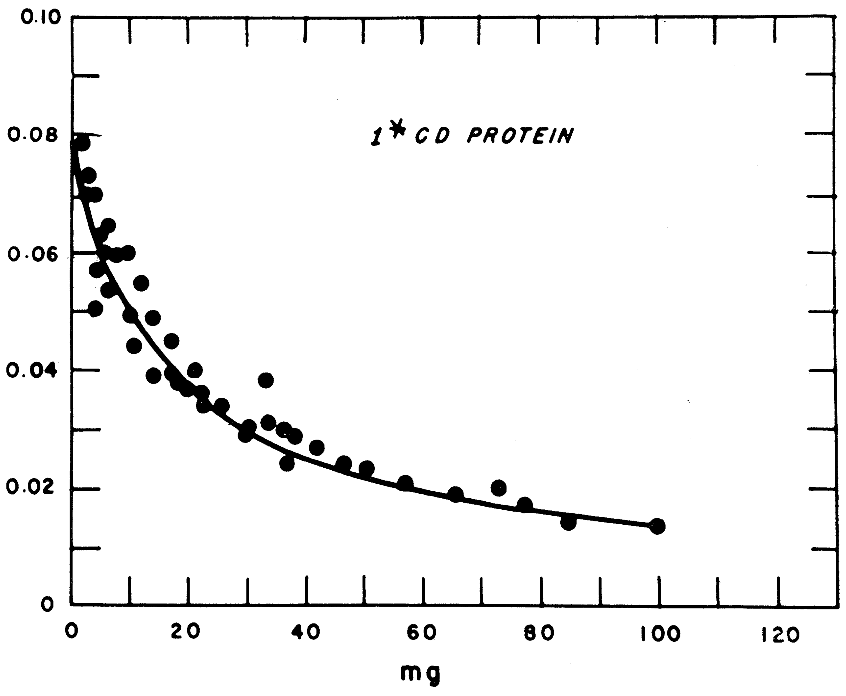

Fig. 4. α, absorption coefficient. α is assumed to be a constant in the exponential treatment, but it is a variable in the hyperbolic treatment. The points are experimental determinations. The line is the predicted variation according to the hyperbolic treatment.

to specific activity at the reference weight or to correct total observed radioactivity at any weight to total radioactivity at the self-absorption of the reference weight. The factor divided by m yields a new factor which can be used to convert total radioactivity at the sample weight to specific activity at the reference weight.

Discussion

There are two equations under consideration in this article.

$$I = I_\infty(1 - 10^{-\alpha m}) \qquad (A)$$

and

$$I = \frac{I_\infty R m}{Rm + I_\infty b} \qquad (B)$$

in the form of

$$\frac{R}{S} = \frac{R}{I_\infty} m + b \qquad (9)$$

where

$$b = R/\sigma$$

Equation A states that the loss of apparent radioactivity with increasing weight of sample is a function of a negative exponential. It assumes that the absorption coefficient α remains constant in spite of the alteration of the spectrum of energy distribution of β particles as the radiation is passed through layers of absorbing substance.

Equation B states that the loss of apparent radioactivity with increasing weight of sample is a hyperbolic function of a rectangular type. It predicts that the absorption coefficient α must vary as the radiation passes through layers of absorbing material (see below).

It is possible to express Eq. A in the form of Eq. 9. The result is:

$$F = \frac{R}{S} = \frac{R}{I_\infty} m + \frac{R}{I_\infty} m \left(\frac{10^{-\alpha m}}{1 - 10^{-\alpha m}}\right) \qquad (11)$$

It can be seen that for a straight line to result from a plot of F against m, the expression

$$\frac{R}{I_\infty} m \left(\frac{10^{-\alpha m}}{1 - 10^{-\alpha m}}\right) = b$$

must be a constant. R and I_∞ are constants, m is a variable; therefore, for the whole expression to be a constant, α must be a variable. By substituting known values for R and I_∞ and solving for α at various m values, we can specify how α, as derived from Eq. A, must vary in order that the observed straight-line plot of F versus m may result. From Eq. 9 and the data of Fig. 1 for protein in the Nuclear Chicago micromil counter we know that at the reference weight of 10 mg

$$F = 1 = 0.068 \times 10 + b$$
$$b = 0.32$$
$$0.32 = \frac{R}{I_\infty} m \left(\frac{10^{-\alpha m}}{1 - 10^{-\alpha m}}\right)$$
$$= 0.068m\left(\frac{10^{-\alpha m}}{1 - 10^{-\alpha m}}\right) \alpha$$
$$= \log\left(\frac{m + 4.7}{4.7}\right) \bigg/ m$$

According to Eq. A, a plot of α versus m should yield a straight line parallel to the m axis. According to Eq. B, a plot of α versus m should yield the curve shown in Fig. 4. The points show the experimental results.

Since it is seen that α in Eq. A is not constant, the physical significance of this α is lost. The equation was derived by treating α as a constant and only has meaning if α *is* a constant. The α in this equation does not represent the absorption coefficient of the material, since it is a variable. The question naturally arises: What is the nature of the true absorption coefficient α^*? An equation for the true absorption coefficient α^* can be derived in the following manner. Referring back to Eq. 7, let us remove the restriction that α is a constant and in-

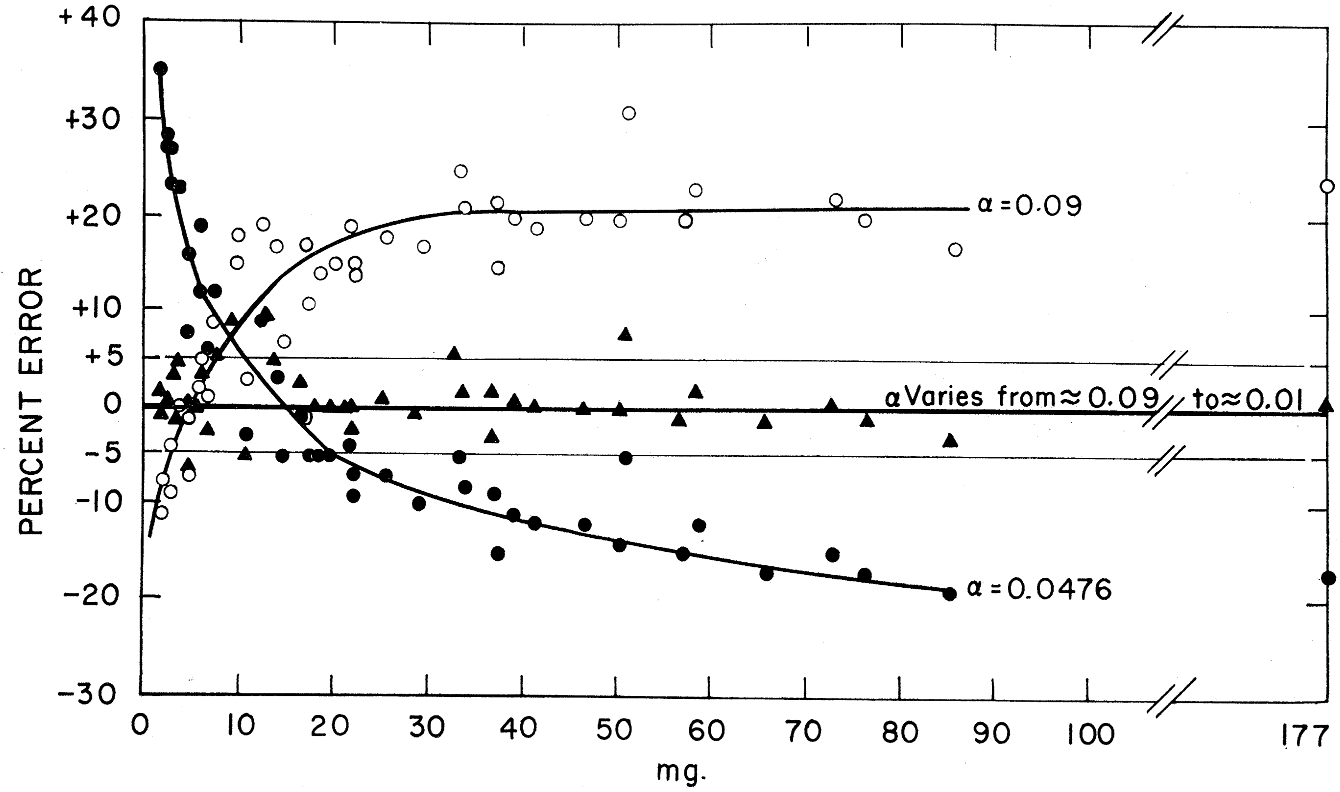

Fig. 5. Percentage of error involved through correcting observed specific activity of protein with factors derived from the exponential treatment ($\alpha = 0.09$ and $\alpha = 0.0476$) and from the hyperbolic treatment, where α varies from 0.09 to 0.01 (Fig. 1). See text for details.

stead ask how must α^* vary to enable us to observe the experimentally determined straight-line function of F versus m. The change in observed activity $\mathrm{d}I$ with each increment of material $\mathrm{d}m$ will be increased by virtue of the specific activity of the material and decreased by virtue of the absorption coefficient, which now will be represented by a function of m, α^*, times the impinging radiation.

$$\frac{\mathrm{d}I}{\mathrm{d}m} = \sigma - \alpha^* I \qquad (12)$$

We have observed from the experimental results that:

$$I = \frac{I_\infty Rm}{Rm + I_\infty b} \qquad (\mathrm{B})$$

$$\therefore \frac{\mathrm{d}I}{\mathrm{d}m} = \frac{I_\infty R(Rm + I_\infty b) - I_\infty R^2 m}{(Rm + I_\infty b)^2} \qquad (13)$$

$$\alpha^* = \frac{\sigma - \dfrac{\mathrm{d}I}{\mathrm{d}m}}{I} \qquad (14)$$

Substituting Eqs. B and 13 in Eq. 14 and utilizing the relation $b = R/b$, and simplifying terms, we find that

$$\alpha^* = \frac{\sigma}{I_\infty} + \frac{\sigma}{\sigma m + I_\infty} \qquad (15)$$

This may be compared to

$$\alpha = \sigma / I_\infty$$

which is true when α is really a constant. It is seen that α^* approaches α as m approaches ∞. At $m = 0$, $\alpha^* = 2\sigma/I_\infty$.

Equation 15 states that the variation of the absorption coefficient is determined by the weight of the sample and by I_∞, which is really a function of the spectrum of energies of the radioactive emission. The actual weight value where I_∞ is experimentally reached is determined by the E_{max} of the spectrum. The actual value of I_∞ itself would be largely determined by the average percentage distribution of high-energy particles. It is to be expected that the energy spectrum would determine the absorption coefficient, and since the energy spectrum is altered as the radiation passes through layers of material, it is most reasonable to conclude that the absorption coefficient does vary with the weight of the material.

The question next arises as to whether in the range of values usually encountered in this work the two different equations might not be so close that no distinction need be made between them. Even if this were true, the simpler form of the second equation might recommend its general use. However, the use of the exponential equation *can* introduce very significant errors, especially if widely different weights of samples are being compared.

The correction factor F was obtained from the exponential equation, on the assumption that the absorption coefficient of 0.09 calculated from considerations given by Libby (*7*) is a constant value. The average absorption coefficient of 0.047 was obtained from the data of Fig. 1 and was similarly used to obtain F from the exponential equation. F was plotted against the weight of the sample, and straight lines were drawn through the nearly linear part of the curves. The correction factors were obtained from these curves as well as from the experimental data where α varies from 0.09 to 0.01 (Fig. 1). Figure 5 shows the error involved in correcting by factors obtained from the three curves. A similar treatment for $BaCO_3$ on the Robinson flow gas counter leads to the results given in Table 1.

It would seem that since the factor does not consistently deviate downwards at decreasing weights, back scattering does not appreciably affect the results at these low values. This may be so for two reasons. (i) The error of weighing in either direction becomes relatively more significant at the weights where back scattering is more appreciable. (ii) At the very low weights there is not an entirely even spread of material, and the average thickness of material through which radiation passes is greater than the thickness for a smooth spread. This would tend to decrease counts, probably in an amount sufficient to counterbalance the increase expected due to back scattering. At any rate the curve appears to maintain its linearity within experimental variations down to the lowest part. The possibility exists that this treatment might apply to the other emitters of beta particles of weak energy distributed over a particular energy range. It has been found that $BaS^{35}O_4$ under counting situation No. 1 yielded a linear plot of the new factor versus m.

In conclusion it should be emphasized that the treatment of radioactivity absorption data by either the exponential or the hyperbolic equation is strictly empirical. The fact that an equation works provides ample basis for its use.

It is to be hoped that a full consideration of the factors involved will someday provide a theoretical basis for the proposed, or for some other more closely fitting, equation.

Summary

A new and simple technique has been developed (*10*) for correcting C^{14} radioactivity measurements for loss of radiation due to self-absorption. Results are discussed which show the applicability of the technique for many different counting situations. It is found that the absorption of radiation follows a hyperbolic law much more closely than an exponential one. Furthermore, the absorption coefficient for the sample itself has been found to be a function of weight rather than a constant, as had been assumed for the derivation of the law of exponential absorption.

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10. The capable technical assistance of Jay Yedvab in this study is gratefully acknowledged. The data for Fig. 3 were kindly supplied by James C. Reid of the National Cancer Institute. Data for phenylalanine-$3C^{14}$ in counting arrangement No. 6 were kindly supplied by Marco Rabinovitz of the National Cancer Institute. I am grateful to James C. Reid of the National Cancer Institute, John L. Stephenson of the National Heart Institute, and W. F. Libby of the Geophysical Laboratory of the Carnegie Institution of Washington for interesting and helpful discussions in the course of this work.

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