A Computer Programme for the Calculation of Radioactivity Data from Liquid-Scintillation Counters fitted with External Standards

By J. M. FELTS* AND P. A. MAYES

Division of Biochemistry, Department of Physiology, Royal Veterinary College, London, N.W. 1

(Received 15 May 1967)

1. A method for transferring data from liquid-scintillation counters to automatic punched-card read-out is described. 2. A method of calibration of an external standard is presented that supplies sufficient information for the correction of quenched data by computer. 3. A computer programme in FORTRAN IV is described that calculates the data from one isotope, or two isotopes counted simultaneously, and that incorporates features for examining the reproducibility of the input data. 4. With the methods described, quench correction of samples containing either ^3H or ^{14}C was accurate until samples were quenched 70%. When the two isotopes were both present in one sample and counted simultaneously, the correction was accurate until the samples were quenched 60%. When quenching was greater than either 70 or 60% respectively, its correction was still possible but with slightly diminished accuracy and with greater variability.

With the developments in engineering that have improved the automatic features of liquid-scintillation spectrometers for the assay of radioactivity, the calculation of data from large numbers of samples has become time-consuming, especially when two isotopes are counted simultaneously and when corrections for quenching must be applied. There is therefore a need for automatic processing of this type of data. We have developed a simple method for the automatic transfer to punched cards of scintillation-counting data, including data derived from the use of an automatic external standard. A computer programme has been written in FORTRAN IV that calculates these data and presents the results as quench-corrected net counts/min. for either one or two isotopes. A number of checks have been incorporated into the programme that test the data for reproducibility and that detect and eliminate gross errors resulting from mechanical or electronic malfunction that occurs, on occasion, with automatic equipment of this type.

Other publications have appeared describing computer programmes or the application of computing methods to the calculation of liquid-scintillation counting data, but these have not provided either for the simultaneous counting of two isotopes, or for the use of an external standard in the correction of quenching (Blanchard, 1963;

Spratt, 1965; Plotka, Stant, Waltz, Garwood & Erb, 1966; Matthijssen, 1966; Braunsberg, 1966). Axelrod, Matthijssen, Goldzieher & Pulliam (1965) described a programme that involved the use of an external standard, but this required handling the data twice during computation and was not recommended for accurate results if quenching exceeded 10%.

EXPERIMENTAL AND RESULTS

Coupling of a liquid-scintillation spectrometer to automatic punched-card output. By means of relays, digital data from a Tri-Carb Liquid Scintillation Spectrometer (model 3314; Packard Instrument Co. Ltd., Wembley) are transferred automatically to cards by using a Printing Card Punch [model 026; International Business Machines (U.K.) Ltd., London, W. 4]. Normally, digital data from the Tri-Carb spectrometer are printed on tape by means of a ten-key adding machine that uses solenoids to activate the keys. Each solenoid requires a relatively large current that allows a small amount to be tapped off from each without interfering with its efficiency. This current is used to activate one side of a contact relay (type 22; Radiospares Ltd., London, W.1). The other side of the relay is used to close the contacts across the corresponding number key of an IBM card punch. Thus each number activated on the ten-key adding machine is simultaneously punched in an IBM card. One additional relay is used to feed in a new card at the end of each 'read-out' sequence. This relay is activated by the 'sample-change' relay of the Tri-Carb spectrometer and closes the contacts of the 'release' key of the card punch. The coupling of the scintillation spectrometer to the card

* Present address: Cardiovascular Research Institute, University of California, School of Medicine, San Francisco, Calif., U.S.A.

punch is inexpensive and has been reliable in continuous operation.

For each sample counted, the digital data from the Tri-Carb spectrometer consisting of the sample number, counting time and the number of counts from each of three channels, including that derived from an external standard, can all be accommodated on one punched card.

Calibration of the external standard. Experiments with the Tri-Carb spectrometer showed that the samples must have the same volume and the same final concentrations of primary and secondary scintillators to ensure reproducibility of counting of the external standard. Each sample contained a standard mixture consisting of 12.0 ml. of a toluene solution containing 1,5-diphenyloxazole (0.4%, w/v) (Packard Instrument Co. Ltd.) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.02%, w/v) (Packard Instrument Co. Ltd.) plus 6.0 ml. of additional solvent. The additional solvent was toluene if lipid-soluble materials were counted and ethanol if more polar materials were involved. We have counted the isotope having β -particles of lower energy in channel 1 whereas the isotope of higher energy was counted in channel 2. Channel 3 was reserved for counting the external standard. In the present instance ^3H was allocated to channel 1 and ^{14}C to channel 2.

To simplify the calculations involved in correcting samples for quenching, it is desirable that a straight-line relationship be established between the counting rate of the external standard in channel 3 and the counting rate of the isotopes in either channel 1 or channel 2. These relationships were established experimentally by counting two series of samples in an all-toluene solvent system. One series contained a standard amount of [^3H]palmitic acid and the other a standard amount of [^{14}C]palmitic acid. The samples also contained 0.1–1.4 ml. of acetone as quenching agent in place of a corresponding volume of toluene. Window size and gain were varied for each channel until straight-line functions were obtained for the relationship between the

counting rate of the external standard and the counting rate of each of the two isotopes (Figs. 1 and 2). The settings selected for counting the external standard in channel 3 were such that neither ^3H nor ^{14}C contributed appreciable counts. In unquenched samples, overlap of ^3H into channel 2 was less than 0.05% whereas overlap of ^{14}C into channel 1 was 38%. For convenience, the same settings were used for counting ^3H alone, ^{14}C alone, or a mixture of the two isotopes. Efficiencies of 38% for ^3H and 55% for ^{14}C were obtained.

The relationship between the fractional quenching of the external standard in channel 3 (X) and the fractional quenching of either ^3H samples in channel 1 or of ^{14}C samples in channel 2 are shown in Figs. 1 and 2 respectively. By using the analysis of variance it was shown that the data in Fig. 2 fitted two distinct regressions ($P < 0.001$). Fig. 3 shows the relationship between the fractional quenching of the external standard (X) and the ratio of ^{14}C counts in channel 1 to ^{14}C counts in channel 2 (Y). As quenching increases, a larger proportion of ^{14}C appears in channel 1 owing to a shift in the energy spectrum. The change in channel 1/channel 2 ratio followed a single exponential function of the fractional quenching of the external standard (X) up to a value of 0.42. At this point there was a significant ($P < 0.001$) break in the relationship that could then be fitted best by a further three-exponential function (Fig. 3). For each exponential, a constant K , analogous to a rate constant, may be calculated (Riggs, 1963):

$$K = \ln 2 / \Delta X = 0.693 / \Delta X \quad (1)$$

Fractional quenching of external standard in channel 3 (X)

Fig. 1. Relationship between fractional quenching of the external standard and fractional quenching of ^3H samples. Tri-Carb spectrometer settings were: channel 1, window 50–550, gain 30%; channel 3, window 1000– ∞ , gain 6%. The curve was fitted by the method of least squares. Each point is the mean of three independent results.

Fractional quenching of external standard in channel 3 (X)

Fig. 2. Relationship between fractional quenching of the external standard and fractional quenching of ^{14}C samples. Tri-Carb spectrometer settings were: channel 2, window 150–600, gain 6%; channel 3, window 1000– ∞ , gain 6%. The curves were fitted by the method of least squares. Each point is the mean of three independent results.

where ΔX is the change in the value of X corresponding to a doubling in the value of Y .
Hence the value of Y corresponding to any value of X is calculated from:

$$Y_x = Y_0 e^{kx} \tag{2}$$

where Y_0 is the value of Y when X is 0.
Thus the curves in Figs. 1, 2 and 3 provide the constants

Fig. 3. Relationship between fractional quenching of the external standard and the ratio of ^{14}C counts channel 1/channel 2. Tri-Carb spectrometer settings were as in Figs. 1 and 2. The curves were fitted by the method of least squares. Each point is the mean of three independent results.

that are needed for the calculation of the quench corrections for counting ^3H alone, ^{14}C alone or a combination of the two. The curves were fitted and the constants were calculated by the method of least squares. The constants remain the same provided that no change is made in the scintillation-counting mixture.

Design of the computer programme. A programme has been written in FORTRAN IV that has been used with an IBM 7090 computer. The input data contained on punched cards consist of three cycles of counting data arranged sequentially. The programme tests the sample number of each card to determine if cards are missing from the sequence or if extra cards are present. Error messages are printed out if the sequence is imperfect. For each sample, three cycles of counts from each channel are examined for gross errors. If there are any variations greater than 50% the appropriate data are rejected. If three or two cycles are accepted, the counts/min. are calculated and averaged. A test is carried out to determine which isotopes are present, the background is subtracted and if there are data from an external standard the counts/min. are corrected for quenching by using the constants originally obtained from Figs. 1, 2 and 3. The counts are corrected further for efficiency of counting. Finally, the data are printed out as corrected counts/min. for each isotope present together with the following information: percentage quenching in channels 1 and 2, ratio of counts in channel 1 to counts in channel 2, percentage s.e.m. for the original counts in each channel, the counting efficiency, the background counts, the counting time and the value of all the constants used for the correction of quenching.

Calculated results from quenched samples. Tables 1, 2 and 3 show how the computer programme corrects for quenching in samples containing ^3H , or ^{14}C alone, or in samples containing both ^3H and ^{14}C .

When ^3H was counted alone (Table 1) correction for quenching was accurate up to the maximum amount of quenching present in the samples, i.e. 74%. When ^{14}C was counted alone (Table 2), correction for quenching was

Table 1. Correction of quenching in samples containing ^3H

Samples were prepared as described in the text. In each case the data were derived from the mean of three independent samples. Although only ^3H was present, the crude counts were corrected for quenching by using a computer programme for calculating the net counts/min. from two isotopes counted simultaneously. This procedure allows the effect of counts in channel 1 (^3H) on the counts in channel 2 (^{14}C) to be ascertained.

Channel 1				Channel 2		
Quenching (%)	Corrected counts/min.	s.d. of corrected counts/min. ($\pm$ %)	Deviation in correction from unquenched samples (%)	Quenching (%)	Corrected counts/min.	% of counts in channel 1 in channel 2
0.0	9296	0.9	0.0	1.0	-2	-0.02
8.4	9262	1.1	-0.4	10.8	1	0.01
18.8	9267	1.7	-0.4	22.9	1	0.01
32.9	9491	2.2	2.1	39.3	2	0.02
49.3	9384	0.6	0.9	58.7	1	0.01
56.3	9314	1.2	0.2	68.1	4	0.04
63.4	9335	1.6	0.4	77.7	3	0.03
66.8	9102	1.0	-2.1	82.3	3	0.03
70.7	9184	1.0	-1.2	87.6	4	0.04
73.9	9317	0.8	0.2	91.9	-2	-0.02

Table 2. *Correction of quenching in samples containing ^{14}C*

Samples were prepared as described in the text. In each case the data were derived from the mean of three independent samples. Although only ^{14}C was present, the crude counts were corrected for quenching by using a computer programme for calculating the net counts/min. from two isotopes counted simultaneously. This procedure allows the effects of counts in channel 2 (^{14}C) on the counts in channel 1 (^3H) to be ascertained.

Channel 1		Channel 2				
Quenching (%)	Corrected counts/min.	Quenching (%)	Corrected counts/min.	s.d. of corrected counts/min. ($\pm$ %)	Deviation in correction from unquenched samples %	% of counts in channel 2 in channel 1
-0.7	-63	0.3	8289	1.8	0.0	-0.76
7.8	-15	10.2	8183	1.9	-1.3	-0.18
17.4	-84	21.3	8206	2.2	-1.0	-1.02
32.4	88	38.6	8132	0.3	-1.9	1.08
48.5	-277	57.7	8265	5.2	-0.3	-3.35
56.3	167	68.1	8193	3.1	-1.2	2.04
63.4	-87	77.7	7867	1.8	-5.4	-1.10
67.3	52	82.9	8225	3.6	-0.8	0.63
70.5	1047	87.3	8587	3.2	3.5	12.19
73.8	69	91.8	10388	3.9	25.3	0.66

Table 3. *Correction of quenching in samples containing both ^3H and ^{14}C*

Samples were prepared as described in the text. In each case the data were derived from the mean of three independent samples. In the columns marked *, the corrected counts/min. are compared with the corresponding unquenched counts/min. in either Table 1 or Table 2.

Channel 1 (^3H)				Channel 2 (^{14}C)			
Quenching (%)	Corrected counts/min.	s.d. of corrected counts/min. ($\pm$ %)	Deviation in correction from unquenched samples* (%)	Quenching (%)	Corrected counts/min.	s.d. of corrected counts/min. ($\pm$ %)	Deviation in correction from unquenched samples* (%)
0.0	9250	1.6	-0.5	1.0	8203	1.5	-1.0
8.4	9330	1.5	0.4	10.8	8255	1.8	-0.4
18.3	9157	2.1	-1.5	22.2	8182	1.7	-1.3
32.3	9264	3.0	-0.3	38.5	8255	1.8	-0.4
49.7	9071	2.8	-2.4	59.2	8299	1.6	0.1
56.3	9424	3.7	1.4	68.1	8277	2.3	-0.1
63.6	8888	9.0	-4.4	78.0	8110	3.4	-2.2
66.9	10101	7.6	8.7	82.4	8122	2.4	-2.0
70.9	9068	4.2	-2.5	87.8	8814	1.0	6.3
73.9	9963	9.6	7.2	91.8	10382	6.8	25.3

accurate until the counts were quenched by approx. 70%. Even when quenching was further increased the correction was accurate to within 5.4% until quenching approached 90%.

When ^3H and ^{14}C were both present in the same sample and were counted simultaneously (Table 3) the accuracy of quench correction in channel 2 (^{14}C) was similar to that for ^{14}C counted alone (Table 2), because the settings for each channel were such that there was negligible spill of ^3H into channel 2. Correction of quenching of ^3H in the presence of ^{14}C was accurate until quenching approached 60%. Between 60 and 74% quenching, the corrected counts deviated by a

maximum of 8.7% from the unquenched samples. These results were obtained with approximately the same activity of ^3H and ^{14}C present in each sample. Clearly, increasing the $^{14}\text{C}/^3\text{H}$ ratio in a sample would increase the relative inaccuracy of correcting for quenching in channel 1. The extreme case is when no ^3H is present and this is illustrated by the results for ^{14}C in Table 2, which have been calculated as if both ^3H and ^{14}C were present. Even under these extreme conditions, provided that the quenching was not higher than 67% in channel 1, the proportion of the counts in channel 2 affecting the counts in channel 1 only amounted to a maximum of 3.4%. Ideally it is desirable to have more

^3H than ^{14}C activity in a sample and to restrict quenching to less than 60% in channel 1.

We thank Professor E. C. Amoroso, F.R.S., and Dr F. A. Holton for criticizing the manuscript. The technical assistance of Miss Tazuko Hirai, Miss Helen Toomey, Mr J. Gunner, Mr I. Kaye and Mr H. Lowe is gratefully recognized. The facilities for computing afforded by Imperial College, London, are also acknowledged. A copy of the programme with instructions for use may be obtained from the authors. The work was supported by U.S. Public Health Service Grant HE06285 and by the Agricultural Research Council. J.M.F. is a recipient of a U.S. Public Health Service Career Development Award.

REFERENCES

- Axelrod, L. R., Matthijssen, C., Goldzieher, J. W. & Pulliam, J. E. (1965). *Acta endocr., Kbh.*, Suppl. 99, p. 7.
- Blanchard, F. A. (1963). *Int. J. appl. Radiat. Isotopes*, **14**, 213.
- Braunsberg, H. (1966). *Biochem. J.* **101**, 44p.
- Matthijssen, C. (1966). *Analyt. Biochem.* **15**, 382.
- Plotka, E. D., Stant, E. G., Waltz, F. A., Garwood, V. A. & Erb, R. E. (1966). *Int. J. appl. Radiat. Isotopes*, **17**, 637.
- Riggs, D. S. (1963). *The Mathematical Approach to Physiological Problems*, pp. 120–167. Baltimore: The William and Wilkins Co.
- Spratt, J. L. (1965). *Int. J. appl. Radiat. Isotopes*, **16**, 439.