A COMPUTER METHOD FOR ANALYSIS OF RADIOACTIVITY DATA FROM SINGLE AND DOUBLE LABELED EXPERIMENTS

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SUMMARY

A generally applicable program has been devised for the machine processing and plotting of radioactivity data. The program can be used with single or double labeled experiments; it includes numerous options which increase its flexibility and facilitate optimization of data presentation.

The digital computer can be an extremely versatile and efficient tool for biological research. As computers become more accessible, their usefulness extends beyond specialized problems to routine applications involving long, complex or reiterative calculations (1). The availability of peripheral hardware, such as computer-run plotters, further increases the usefulness of computers for routine analysis of data. This report presents a program (2) which permits a widely available computer-plotter system to perform, in a single run, all operations from the reading of counts collected by a radioactivity counter to the calculation of results and their presentation in graph form. The results obtained are numerical and percent values for two isotopes, numerical and normalized isotope ratio, numerical and percent error margins of the ratio, quenching as determined by the channels method, plus the same parameters calculated after combining samples as needed to reduce statistical uncertainty below a specified level.

The program is economical to run (typical cost $9.50 for processing and plotting the results of a double labeled experiment involving 1000 samples) and requires no familiarity with computer operations beyond card punching. Since *de novo* development of such a program requires a substantial initial investment (ca. $500) and considerable programming skills, we want to publicize the existence of our program and make it available to other workers. Copies of the program and detailed instructions for its use may be obtained from F. C. Kafatos.

This program was developed on an IBM 360-65 computer with associated Calcomp plotter (3). The programming language is Fortran IV (4); extensive comments are included to facilitate use of the program by other workers. One of us (EWY) did the actual programming. We developed the program for use with H^3- and C^{14}-labeled acrylamide gels, sectioned into slices and counted by liquid scintillation; it can be adapted, with only trivial changes, for use with any single or double labeled experiment. Indeed, it could be also modified for experiments involving measurements of optical absorption, enzymatic activity, conductivity etc.

The program was designed for use with minimal effort in routine experiments (Table I, Figure 1). In addition, its various options permit one to recalculate or replot the data so as to maximize the information content and clarity of the presentation. The entire experiment may be submitted for alternative presentations, or a subset of the data (e.g. a specific MW range in an SDS-acrylamide gel) can be selected for calculation and presentation.

TASKS PERFORMED BY THE PROGRAM

1. Calculation of radioactivity in each sample. An example of the data input and the computer print output for an acrylamide gel is given in Table I. The counts collected for each sample (slice) by up to 3 scintillation counter channels are recorded on a punch card either manually or by a tape-card converter if the scintillation counter is equipped with tape puncher. The data cards are submitted to the computer, together with a copy of the program (a compiled "object deck") and instruction cards (specifying options to be used in calculating and plotting, background counts in each channel, crossover of C^{14} into the H^3 channel, and time, in minutes, over which the counts in each slice were collected). The computer output presents: (a) H^3 cpm corrected for background and C^{14} crossover (net H^3 cpm), (b) net H^3 cpm as percent of total H^3 in the gel, (c) C^{14} corrected for background (net C^{14} cpm), and (d) net C^{14} cpm as percent of total C^{14} in the gel. The percentage calculations facilitate comparison between experiments involving widely different levels of radioactivity.

2. Calculation of H^3/C^{14} ratios. The ratio of net H^3 cpm/net C^{14} cpm is calculated for each slice. This ratio is also normalized against the overall ratio for the gel. The normalized ratio has two main advantages: it identifies deviations from identity of the two isotope profiles (ratio 1.0), and it facilitates comparison of experiments regardless of the level of individual isotope incorporation or efficiencies of counting.

3. Error analysis. The computer calculates the 95% confidence level for counting statistics (7,8). This is an index of how closely the counts collected correspond to the true value of counts in the sample; for each isotope there is 95% confidence that the true value of radioactivity lies within a computed range $[\pm 1.96(\frac{\text{cpm gross}}{\text{total time}} + \frac{\text{cpm background}}{\text{total time}})^{1/2}]$. The error of the H^3 cpm/C^{14} cpm ratio is the sum of the errors for the two isotopes; it is expressed both as a numerical value and as a percentage.

TABLE I

Computer Calculations

DAY 8-9 36H ACT. D , 36H CONTROL

A

TOTAL H-3 ON GEL= 27022. TOTAL C-14 ON GEL= 6100. H3/C14= 4.43

	H3	H3-BKG	XOVER	H3-BKG -XOVER	PCT H3	C14	C14-BKG	PCT C14	H3/C14 RAW	H3/C14 NORMAL	ERROR	PERCENT	C/C
1	95.	72.	9.	63.	0.23	58.	45.	0.73	1.41	0.32	0.23	16.12	2.40
2	71.	49.	9.	40.	0.15	57.	43.	0.71	0.92	0.21	0.16	17.60	2.20
3	78.	56.	13.	43.	0.16	76.	63.	1.03	0.69	0.16	0.11	15.64	1.92
4	96.	74.	15.	59.	0.22	88.	75.	1.22	0.79	0.18	0.11	14.12	1.85
5	131.	108.	23.	85.	0.32	128.	115.	1.88	0.75	0.17	0.09	11.63	1.89
6	170.	147.	25.	123.	0.45	136.	123.	2.02	1.00	0.22	0.11	10.62	1.84
7	203.	181.	31.	150.	0.56	166.	153.	2.51	0.98	0.22	0.09	9.58	1.83
8	261.	239.	44.	195.	0.72	234.	221.	3.63	0.88	0.20	0.07	8.16	1.86
9	212.	190.	40.	150.	0.55	214.	200.	3.29	0.75	0.17	0.07	8.84	1.80
10	264.	242.	41.	201.	0.74	218.	205.	3.36	0.98	0.22	0.08	8.29	1.89
11	330.	308.	37.	271.	1.00	197.	184.	3.01	1.48	0.33	0.12	8.09	1.93
12	502.	480.	42.	438.	1.62	221.	208.	3.41	2.11	0.48	0.15	7.12	1.85
13	285.	263.	32.	230.	0.85	175.	162.	2.66	1.42	0.32	0.12	8.67	1.86
14	251.	229.	28.	201.	0.74	152.	139.	2.28	1.44	0.33	0.13	9.32	1.95
15	387.	365.	37.	328.	1.21	199.	186.	3.04	1.76	0.40	0.14	7.78	1.82
16	469.	446.	35.	411.	1.52	190.	177.	2.89	2.33	0.53	0.18	7.58	1.84
17	620.	597.	38.	559.	2.07	203.	190.	3.11	2.95	0.67	0.21	7.03	1.91
18	732.	710.	35.	674.	2.50	190.	177.	2.90	3.81	0.86	0.27	6.97	1.88
19	1030.	1007.	42.	966.	3.57	221.	208.	3.41	4.65	1.05	0.29	6.24	1.86
20	399.	376.	31.	345.	1.28	168.	155.	2.54	2.23	0.50	0.18	8.16	1.97
21	337.	315.	33.	281.	1.04	179.	166.	2.72	1.69	0.38	0.14	8.28	1.88
22	351.	328.	33.	295.	1.09	177.	164.	2.68	1.80	0.41	0.15	8.25	1.88
23	300.	278.	26.	252.	0.93	145.	132.	2.17	1.90	0.43	0.17	9.07	1.88
24	407.	384.	27.	357.	1.32	148.	134.	2.20	2.66	0.60	0.23	8.48	1.95
25	469.	446.	30.	416.	1.54	165.	151.	2.48	2.75	0.62	0.22	7.95	1.80
26	596.	574.	29.	545.	2.32	158.	144.	2.37	3.77	0.85	0.29	7.73	1.90
27	1085.	1063.	36.	1028.	3.80	191.	178.	2.92	5.77	1.30	0.38	6.54	1.93
28	1559.	1537.	36.	1501.	5.55	192.	179.	2.93	8.39	1.89	0.52	6.20	1.92
29	1029.	1007.	37.	970.	3.59	198.	185.	3.03	5.25	1.19	0.34	6.50	1.93
30	1080.	1057.	44.	1013.	3.75	235.	221.	3.63	4.57	1.03	0.28	6.06	1.95
31	1915.	1893.	43.	1850.	6.85	227.	214.	3.51	8.63	1.95	0.49	5.65	1.90
32	1670.	1648.	27.	1621.	6.00	147.	134.	2.20	12.38	2.73	0.83	6.86	2.00
33	1545.	1523.	20.	1503.	5.56	114.	101.	1.66	14.83	3.35	1.14	7.71	2.03
34	874.	852.	17.	835.	3.09	96.	83.	1.36	10.04	2.27	0.89	8.86	2.05
35	1070.	1047.	15.	1032.	3.82	89.	76.	1.25	13.53	3.05	1.21	8.93	1.95
36	435.	413.	13.	400.	1.48	76.	63.	1.03	6.35	1.43	0.68	10.74	1.89
37	501.	479.	15.	463.	1.71	90.	77.	1.26	6.04	1.36	0.59	9.83	1.97
38	384.	362.	11.	350.	1.30	70.	56.	0.93	6.20	1.40	0.70	11.35	1.96
39	565.	542.	15.	528.	1.95	87.	74.	1.21	7.15	1.61	0.70	9.79	1.89
40	634.	612.	13.	599.	2.22	78.	65.	1.07	9.21	2.08	0.93	10.08	2.18
41	1062.	1040.	16.	1024.	3.79	95.	82.	1.34	12.53	2.83	1.09	8.71	1.94
42	1531.	1509.	20.	1488.	5.51	114.	101.	1.65	14.77	3.33	1.14	7.73	2.10
43	1685.	1662.	21.	1641.	6.07	120.	107.	1.75	15.33	3.46	1.15	7.48	2.01
44	1133.	1111.	16.	1095.	4.05	95.	82.	1.35	13.32	3.01	1.15	8.63	2.00
45	236.	213.	8.	205.	0.76	53.	40.	0.65	5.15	1.16	0.71	13.75	2.34
46	137.	115.	4.	111.	0.41	34.	20.	0.34	5.39	1.22	0.99	18.32	2.44
47	87.	65.	2.	63.	0.23	23.	10.	0.16	6.25	1.41	1.48	23.60	5.56
48	45.	22.	2.	20.	0.08	22.	8.	0.14	2.40	0.54	0.68	28.30	3.86
49	29.	7.	1.	6.	0.02	19.	5.	0.09	0.98	0.22	0.34	34.12	-9.17
50	23.	1.	1.	1.	0.00	22.	9.	0.15	0.11	0.03	0.04	34.62	4.09
51	25.	3.	2.	1.	0.00	21.	8.	0.14	0.16	0.04	0.05	33.88	3.46

DAY 8-9 36H ACT. D , 36H CONTROL

B

TOTAL H-3 ON GEL= 27022. TOTAL C-14 ON GEL= 6100. H3/C14= 4.43

	H3	H3-BKG	XOVER	H3-BKG -XOVER	PCT H3	C14	C14-BKG	PCT C14	H3/C14 RAW	H3/C14 NORMAL	ERROR	PERCENT	C/C
4	85.	63.	11.	51.	0.19	69.	56.	0.92	0.91	0.21	0.09	9.74	1.85
6	153.	128.	24.	104.	0.39	132.	119.	1.95	0.87	0.20	0.07	8.25	1.84
7	203.	181.	31.	150.	0.56	166.	153.	2.51	0.98	0.22	0.09	9.58	1.83
8	261.	239.	44.	195.	0.72	234.	221.	3.63	0.88	0.20	0.07	8.16	1.86
9	212.	190.	40.	150.	0.55	214.	200.	3.29	0.75	0.17	0.07	8.84	1.80
10	264.	242.	41.	201.	0.74	218.	205.	3.36	0.98	0.22	0.08	8.29	1.89
11	330.	308.	37.	271.	1.00	197.	184.	3.01	1.48	0.33	0.12	8.09	1.93
12	502.	480.	42.	438.	1.62	221.	208.	3.41	2.11	0.48	0.15	7.12	1.85
13	285.	263.	32.	230.	0.85	175.	162.	2.66	1.42	0.32	0.12	8.67	1.86
14	251.	229.	28.	201.	0.74	152.	139.	2.28	1.44	0.33	0.13	9.32	1.95
15	387.	365.	37.	328.	1.21	199.	186.	3.04	1.76	0.40	0.14	7.78	1.82
16	469.	446.	35.	411.	1.52	190.	177.	2.89	2.33	0.53	0.18	7.58	1.84
17	620.	597.	38.	559.	2.07	203.	190.	3.11	2.95	0.67	0.21	7.03	1.91
18	732.	710.	35.	674.	2.50	190.	177.	2.90	3.81	0.86	0.27	6.97	1.88
19	1030.	1007.	42.	966.	3.57	221.	208.	3.41	4.65	1.05	0.29	6.24	1.86
20	399.	376.	31.	345.	1.28	168.	155.	2.54	2.23	0.50	0.18	8.16	1.97
21	337.	315.	33.	281.	1.04	179.	166.	2.72	1.69	0.38	0.14	8.28	1.88
22	351.	328.	33.	295.	1.09	177.	164.	2.68	1.80	0.41	0.15	8.25	1.88
23	300.	278.	26.	252.	0.93	145.	132.	2.17	1.90	0.43	0.17	9.07	1.88
24	407.	384.	27.	357.	1.32	148.	134.	2.20	2.66	0.60	0.23	8.48	1.95
25	469.	446.	30.	416.	1.54	165.	151.	2.48	2.75	0.62	0.22	7.95	1.80
26	596.	574.	29.	545.	2.32	158.	144.	2.37	3.77	0.85	0.29	7.73	1.90
27	1086.	1063.	36.	1028.	3.80	191.	178.	2.92	5.77	1.30	0.38	6.54	1.93
28	1559.	1537.	36.	1501.	5.55	192.	179.	2.93	8.39	1.89	0.52	6.20	1.92
29	1029.	1007.	37.	970.	3.59	198.	185.	3.03	5.25	1.19	0.34	6.50	1.93
30	1080.	1057.	44.	1013.	3.75	235.	221.	3.63	4.57	1.03	0.28	6.06	1.95
31	1915.	1893.	43.	1850.	6.85	227.	214.	3.51	8.63	1.95	0.49	5.65	1.90
32	1670.	1648.	27.	1621.	6.00	147.	134.	2.20	12.38	2.73	0.83	6.86	2.00
33	1545.	1523.	20.	1503.	5.56	114.	101.	1.66	14.83	3.35	1.14	7.71	2.03
34	874.	852.	17.	835.	3.09	96.	83.	1.36	10.04	2.27	0.89	8.86	2.05
35	1070.	1047.	15.	1032.	3.82	89.	76.	1.25	13.53	3.05	1.21	8.93	1.95
37	468.	446.	14.	432.	1.60	83.	70.	1.15	6.18	1.40	0.47	7.64	1.97
39	474.	452.	13.	439.	1.62	78.	65.	1.07	6.74	1.52	0.53	7.83	1.89
41	848.	826.	15.	811.	3.00	86.	73.	1.20	11.06	2.50	0.77	6.92	1.94
42	1531.	1509.	20.	1488.	5.51	114.	101.	1.65	14.77	3.33	1.14	7.73	2.10
43	1685.	1662.	21.	1641.	6.07	120.	107.	1.75	15.33	3.46	1.15	7.48	2.01
44	1133.	1111.	16.	1095.	4.05	95.	82.	1.35	13.32	3.01	1.15	8.63	2.00
51	83.	61.	3.	58.	0.21	28.	15.	0.24	3.98	0.90	0.54	13.63	

(A): Photograph of non-collapsed computer printout. The 51 gel slices of the experiment described in Fig. 1 were counted for 10 minutes each and the data submitted to the computer. Background 10-minute counts were 224 (H^3), 131 (C^{14}), and 95 ($C^{14'}$); carryover of C^{14} into H^3 was 20%. The raw counts for slice 1 were 945 (H^3), 578 (C^{14}) and 281 ($C^{14'}$).

(B): Collapsed computer printout.

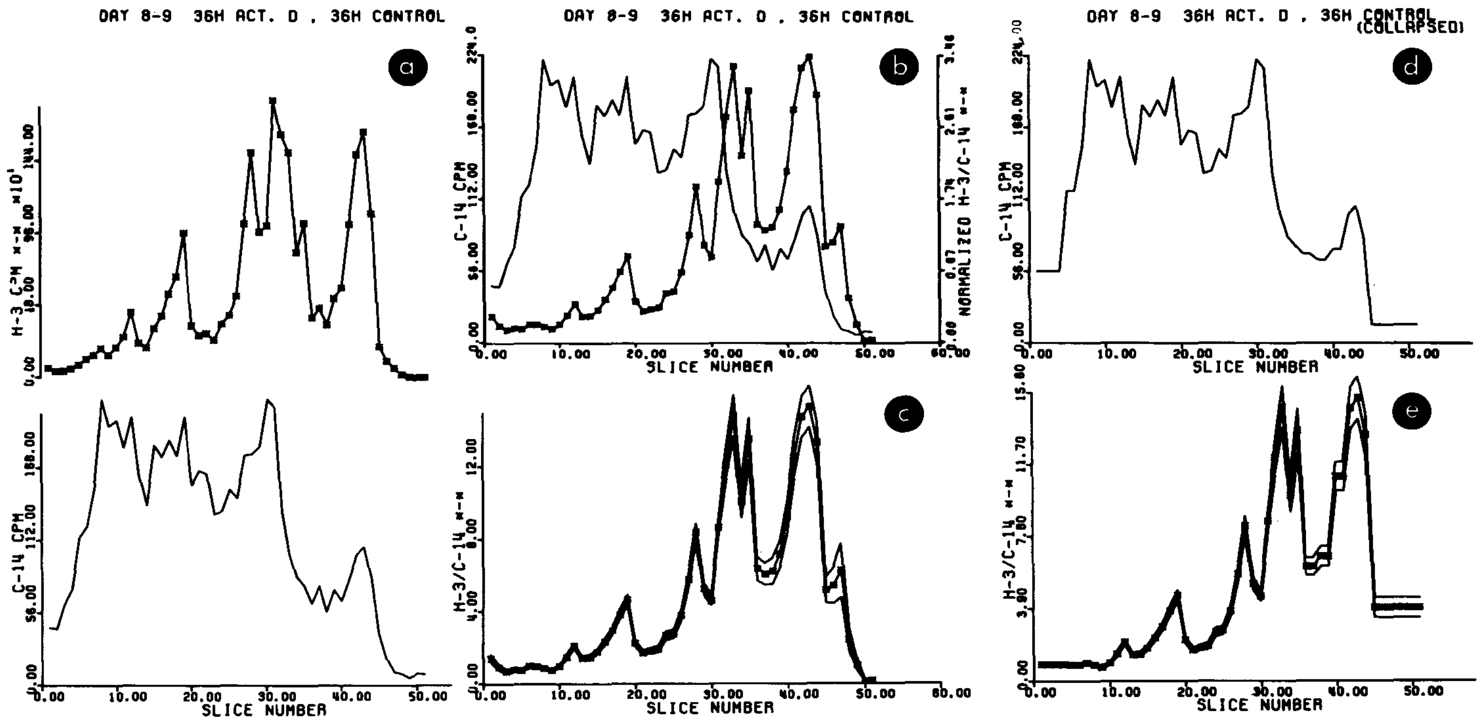

Figure 1. Computer drawn plots. Wing tissue from developing silkmoths, *Antheraea polyphemus* in the 8-9 day of adult development was cultured *in vitro* (2, 5, 6) for 36 hours with or without 60 μg/ml actinomycin D. The actinomycin D-treated tissue was then labeled for 1 hour with H^3-leucine (200 μC/ml, 3.75 μg/ml) and the control tissue with C^{14}-leucine (10 μC/ml, 3.75 μg/ml) for 1 hour. Aliquots of both tissues were combined and the total proteins fractionated by electrophoresis on a 0.5% SDS-10% acrylamide gel (2). The gel was sectioned into 1 mm slices and counted (6) in a 3-channel liquid scintillation counter. The noncollapsed and collapsed computer printouts are shown in Table IA and IB, respectively. (a): "split plot" showing net H^3 and C^{14} count profiles. (b): "combined plot" showing the net C^{14} counts profile and the normalized H^3/C^{14} ratio. (c): "error plot," showing the H^3/C^{14} ratio plus the 95% confidence range. (d): "single plot" of the collapsed net C^{14} count profile. (e): collapsed error plot.

4. Quench calculation. For a comparison of ratios among slices, it is sufficient that quenching be constant, i.e., that the ratio of counts collected at one fixed setting to counts collected at another fixed setting for the same isotope be constant. In these experiments two different C^{14} settings were used. The $C^{14}/C^{14'}$ channels ratio is calculated for each slice.

5. Collapsed computations. Having completed the computation of results for each slice, the computer now scans the % error of the ratio for each slice. If the % error is greater than 10% (or any other predetermined level), the counts of that slice are added to those of the following, and a new ratio and error are computed for the summed counts. Summation continues until the error is reduced to less than 10%. The average cpm for the slices is printed against the number of the last slice summed, and the computer proceeds to the next slice. Summation or "collapsing" smooths the curves by eliminating inexact values; in plotting, the computer employs the averaged values for all slices included in a particular summation. Thus, collapsed regions are easily identifiable as straight horizontal segments (Figure 1d,e).

6. Graph plotting. This program permits plotting of up to 10 collapsed and 10 non-collapsed graphs in a single run. The parameters that can be plotted are (a) net H^3 cpm, (b) net C^{14} cpm, (c) percent H^3, (d) percent C^{14}, (e) H^3/C^{14} ratio and (f) normalized H^3/C^{14} ratio. Any of these parameters may be plotted singly (Figure 1d). Alternatively, any two parameters may be superimposed on the same abscissa and ordinate (Figure 1b); or a "split plot" may be drawn, with two parameters plotted on the same abscissa but on separate ordinates (Figure 1a).

In addition the program can produce an "error plot" by drawing the H^3/C^{14} ratio profile with asterisks and, on either side of that profile, the 95% confidence limits with a plain line (Figure 1c, e). Error plots permit a quick assessment of the significance of variations in the ratio; thus, in Figure 1c all ratio peaks are seen to be significant with the exception of the one farthest to the right (slice 47).

OPTIONS AVAILABLE IN THE USE OF THIS PROGRAM

Several options of specifying alternatives for computation or plotting exist in the program. Selection is accomplished by instruction cards. If no special instructions are given, the computer proceeds in a standard way. This arrangement has the double advantage of simplifying routine processing of data while permitting flexibility when needed. A summary of the options is given in Table II. If desired, options could be easily eliminated altogether from the program, thus making it even more economical than in its present form.

In sum, this program has proven invaluable in our research. It represents a fast, accurate and economical alternative to laborious manual calculating and

TABLE II

Optional Specifications

Number of first slice entered	The abscissa always begins at 0. Data slices are positioned on the abscissa depending on the number assigned to the first slice.
Length of abscissa	Twelve inches maximum.
Length of ordinate	Eight inches maximum.
Abscissa scale	Number of slices to be plotted per inch.
Ordinate scale	Change in value to be plotted per inch. Different scales may be specified for each parameter plotted.
Ordinate zero	The ordinate need not begin at 0.0; a different value may be specified as zero for each parameter plotted.
% error limit	The standard used for collapsing slices. In the standard settings, 10% is assumed for double labeled experiments and 5% for single labeled.
Plotting	Any one of the six parameters may be plotted singly, or any two in a combined or split plot. Error plots cannot be included in combined or split plots.

plotting, and it helps optimize data presentation. Indeed, it permits research projects which would not be otherwise undertaken because of massive requirements for data analysis.

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