

Chapter 20

On-line Scintillation Counters in a Drug Development Environment

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INTRODUCTION

In 1971 at the second Symposium of the Group held in Brighton, we presented a paper on a computerized approach to the use of external standardization in the calculation of dpm values of biological samples.¹ During the subsequent six years, considerable advances have been made in various methods of data processing, and today I doubt if there are many in this audience who do not have either access to a computer or a dedicated package supplied by a particular manufacturer. Possibly, some of you have had to make the decision as to the type of system you require.

At Hoechst about four years ago the decision was, to some extent made for us. In setting up the pharmaceutical research laboratories at Milton Keynes, one of the plans was to have a minicomputer which would serve various user groups and where possible an on-line mode would be employed. One such group was what is known today as Counting Services, a section responsible for all liquid scintillation counting on site, but whose largest user of β -emitting isotopes is the Drug Development Group. My presentation will therefore be on the topic of on-line scintillation counters in a drug development environment.

METHOD

When the research laboratories were set up four years ago, we were in a position to give some thought to the type of system which would cover our basic needs. In Fig. 1 are shown the various procedures which would be required to be covered by an on-line system. The majority of the radioactive work within drug development is concerned with studies in laboratory animals and man. This work can be subdivided broadly into two sections, namely studies to obtain excretion data and those to provide tissue distribution data. Another aspect of the work involves data from TLC or HPLC where chemical analyses are often expressed in the form of histograms, and in addition, the computer can be of immense help in protein-binding studies. All these applications will be described in more detail later on.

1. Animal studies
 - (a) Excretion data
 - (b) Tissue distribution
2. TLC and HPLC output
3. Protein binding studies

Fig. 1 Procedures requiring radioactivity analyses.

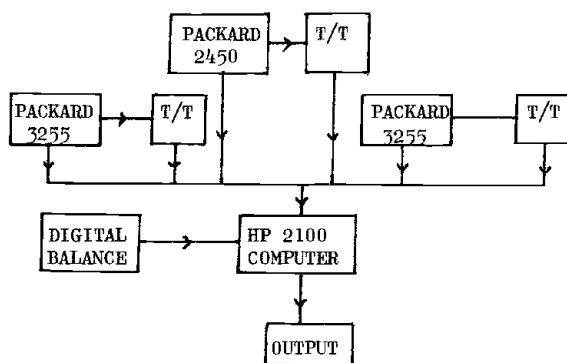


Fig. 2 The basic system

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ON, COUB
MACHINE NUMBER 3
COUNTER STOPPED YE
NUMBER OF TRAYS = 01
ENTER CALIBS + OWNER TRAY 5 1, 1, 0, C
HEADER 14C- CONTENT BATH SPA WATER
COUNTER ON
  
```

Fig. 3 Data fed to computer after counting overnight.

The design of the basic system is shown in Fig. 2. Initially we purchased a Packard 2450 and more recently, two 3255 models. All three scintillation counters can be run either on- or off-line. When the counters are on-line the teletypes can be used independently; we also have a top pan balance on-line to the computer for the storage of sample weights. The importance of this will be seen later. Data output from the computer, a Hewlett-Packard 2100, can be via a line-printer but normally it is on one of the laboratory teletypes.

One possible shortcoming of the computerized approach to data handling is that people often blindly accept the values which are typed out at the end of the computations. Although our system was designed to be versatile and easy to use and with a minimum possibility of operator error, it was also designed to give valid answers. Normally samples are counted overnight and the cpm values stored. The following morning the required information is fed to the computer (Fig. 3).

The program title is COUB, the machine number in this example was 3 and the number of trays counted was 1. The samples in tray 5 were counted in scintillator 1, were single-labelled (1), the label being ¹⁴C (C), and the user code was 0. The computer types the message 'COUNTER ON', and within about 5 s the results are printed out (Fig. 4) and the scintillation counter is again ready for use.

The dpm values are calculated using an AES channels ratio method and a sixth-order polynomial. A retention code is assigned to each set of results, to be used if required for further computations.

The quenched standards used to calculate the sample dpm values are counted most nights in position 1. If for some reason they are not, then we use previously counted standard results, which have been stored. As a check, each set of results of freshly counted standards is compared with those stored and a suitable message is typed at the terminal if significant differences are found.

As a further check on the accuracy of the computer dpm values, a number of samples (about 10% of each set) are internally standardized and recounted in turret or tray position 2. After counting, the information shown in Fig. 5 is input to the computer. The computer requires to know the machine in which the spiked sample was originally

14C CONTENT BATH SPA WATER WEDNESDAY 24 AUGUST 77 (236)

MACHINE NO 1 LABELS 1

C A R B O N 1 4

TRAY	SAM	AES	CPM	DPM	ZERR	EFF
5	0	.581	19	0\$\$\$\$\$.7634
5	1	.578	6735	8809	1.2	.7624
5	2	.578	6946	9085	1.2	.7625
5	3	.587	6982	9104	1.2	.7648
5	4	.599	14177	18449	1.0	.7674
5	5	.600	14239	18527	1.0	.7676
5	6	.590	14145	18448	1.0	.7657
5	7	.586	20629	26948	1.0	.7648
5	8	.584	21106	27592	1.0	.7643
5	9	.581	20571	26921	1.0	.7634

DATA RETAINED UNDER CODE FA FOR ONE WEEK

STOP

Fig. 4 Results from counting example

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ON, SPIK
MACH NO # 1
TRAY: SAM NO#3,15
DPM CA*10 1747
EFF(CA)=46.75%
TRAY: SAM NO#4,9
EFF(CA)=77.59%
STOP
    
```

Fig. 5 Input to computer for internal standardization of computer dpm values.

1. Preparation of quenched standards
2. Machine variation
3. Sample counting variation
4. Sample curve fitting

$$\text{Total error}^2 = 1^2 + 2^2 + 3^2$$

Fig. 6 Factors which may be included in the error factor.

counted and its original tray (or turret) number and sample position. The dpm value of the added internal standard is typed and the efficiency of the sample is calculated and printed. The counting efficiency value by spiking is noted against the value for external standardization and it is up to the user to decide if agreement between them is acceptable.

You will remember that on the dpm print-out there was an error figure. We believe that this is essential, especially to satisfy the requirements of drug regulatory authorities. In Fig. 6 are shown the factors which could be included in the error factor. Since any error arising from sample curve fitting is accounted for by internal standardization and by testing every sample/scintillator combination with the appropriate set of quenched standards¹ factor 4 is not included in the total error figures.

$$\text{ERROR IN CPM} = \frac{\text{TOTAL COUNTS}}{\text{TIME}}$$

$$\text{ERROR IN CPM}^2 = \frac{\text{TOTAL COUNTS}}{\text{TIME}^2}$$

$$\text{TOTAL CPM ERROR}^2 = \text{ERROR CPM}^2 + \text{ERROR BG}^2$$

$$\text{ERROR DPM}^2 = F^2 (\text{ERROR CPM}^2 + \text{ERROR BG}^2)$$

Fig. 7 Calculation of sample counting variation.

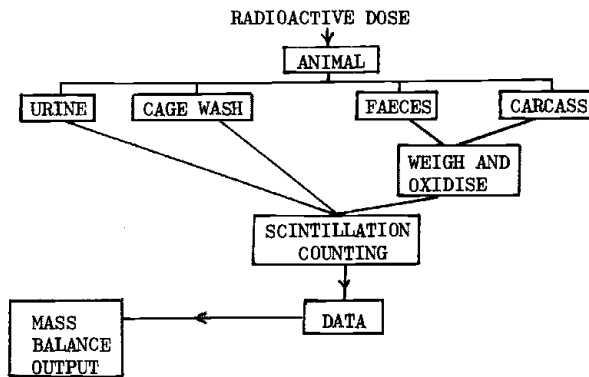


Fig. 8 Stages in a typical animal study.

The error in the preparation of quenched standards we calculated by taking the percentage error figures obtained by Wellcome for their micrometer syringe and by the Radiochemical Centre, Amersham, Bucks., for ^{14}C - and ^3H -hexadecane. Machine variation was estimated by counting each set of quenched standards ten times. The sample counting variation was calculated as shown in Fig. 7, where F is derived from the AESR curve.

We have now obtained, we hope, the correct dpm values for our biological samples. These values are stored and used if required in any further computations.

APPLICATIONS

Some examples of how we use this on-line system in our routine work will now be presented. First as a major example, its use in animal excretion studies will be described. The various stages of a typical animal study are shown in Fig. 8. An animal is dosed with a radioactive compound and the various samples shown are collected. The scintillation counting of urine and cage washings is no problem but the combustion of faecal and carcass homogenates can be very time consuming in terms of weighing, oxidizing and calculation of results. For this reason, we have the on-line digital balance included in our system which will give a set of weights under a unique retention code. Following scintillation counting, the generation of data is as previously described. To arrive at a mass balance output, we use the type of logic shown in Fig. 9. The oxidizer program takes into consideration the efficiency of the sample oxidizer and there are programs for the other routines. We end up with a total dpm value for that sample which could be the 6 h urine from rat 1 or the cage washings from dog 2. This value is stored and a unique retention code is assigned to it. When we have all the required data from the experiments we can obtain a mass balance print-out of the results (Fig. 10).

A further application from animal studies can be seen when tissue distribution data are required. Having obtained dpm values from the combustion and counting of plasma and tissue samples the information shown in Fig. 11 can be fed to the computer. Since no file code has been previously allotted, the computer now assigns one (in this case

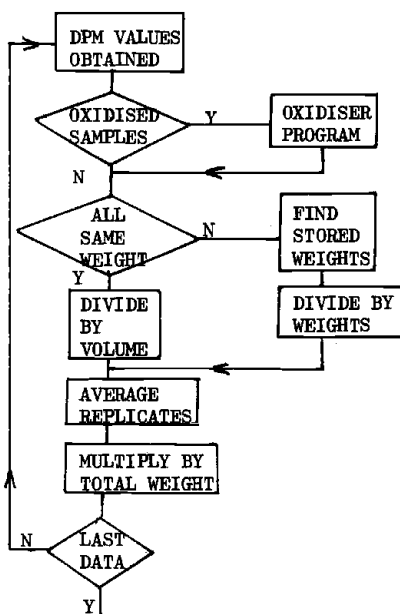


Fig. 9 Flow chart showing the logic used to obtain a mass balance output.

MASS BALANCE PROGRAM
HP 505 RAT P.O. STUDY 1 MG/KG

ANIMAL NO 1

HOURS	URINES		FAECES		CAGE WASH		CARCASS	
	PER	ERR	PER	ERR	PER	ERR	PER	ERR
6	10.4	.2						
24	32.7	.4	34.2	.3				
48	40.1	.5	49.9	.5				
72	42.0	.5	53.8	.6				
96	42.2	.5	55.7	.7	.5	.0	.3	.0

TOTAL RECOVERY 98.7 1.0

Fig. 10 Mass balance print-out of results.

ON,FAPP

FILE NAME* NO
FILE NAME DBABG
DIV 154 2 HR 2ND LABEL
1,.2352,.2307,4.
7,.0227,.0333,.056
2,.3327,.4322,6.
10,.5294,.6456,4.77
15,.2664,.2822,.5486

Fig. 11 Program for obtaining tissue distribution data.

*ON,PKBB

ENTER TOTAL MICRO C1 14.95

SPEC ACT? 18700

TOT WEIGHT? 396

FILE CODE? IG,AB

HEADER

DIV 154 2 HR 2ND LABEL

	DPM	% PER GRM	% ACT.	PPM	ACT/PLAS	TOT ACT	ACT	OX WT
PLASMA								
	14808.8	.1897	.759	1.5167	.000	.1134	.0067	.2352
	22349.7	.2919	1.168	2.3336	.000	.1746	.0101	.2307
	18579.2	.0360	.963	1.9251	.000	.1440		
ADRENAL								
	2271.6	.3015	.017	2.4105	1.252	.0025	.0010	.0227
	2252.5	.2038	.011	1.6294	.846	.0017	.0010	.0333
	2262.1	.0378	.014	2.0200	1.049	.0021		
BLOOD								
	29689.2	.2689	1.613	2.1496	1.117	.2412	.0134	.3327
	48406.1	.3375	2.025	2.6979	1.401	.3027	.0218	.4322
	39047.6	.0453	1.819	2.4237	1.259	.2719		
BRAIN								
	3123.1	.0178	.085	.1421	.074	.0127	.0014	.5294
	3712.7	.0173	.083	.1385	.072	.0124	.0017	.6456
	3417.9	.0026	.084	.1403	.073	.0125		
EYE								
	2744.5	.0310	.017	.2482	.129	.0025	.0012	.2664
	3425.4	.0366	.020	.2924	.152	.0030	.0015	.2822
	3085.0	.0051	.019	.2703	.140	.0028		

Fig. 12 Typical output from tissue distribution program

14C CONTENT BATH SPA WATER
CARBON 14

NO	PER	ERR	Ø	5	1Ø	15	2Ø
1	4.8	.1	-----	(+)			
2	1Ø.1	.1	-----	-----	+		
3	9.9	.1	-----	-----	+		
4	9.2	.1	-----	-----	+		
5	2.6	.Ø	-----	+			
6	4.2	.1	-----	+			
7	3.9	.Ø	-----	+			
8	7.9	.1	-----	+			
9	15.1	.3	-----	-----	-----	(+)	
1Ø	6.6	.1	-----	-----	+		
11	2.7	.Ø	-----	+			
12	2.5	.Ø	-----	+			
13	5.7	.1	-----	-----	(+)		
14	1Ø.6	.2	-----	-----	-----	+	
15	1.8	.Ø	-----	+			
16	2.5	.Ø	-----	+			

Fig. 13 Results of analysis of ¹⁴C content of Bath Spa water expressed as percentage distribution values and in histogram form. The brackets at the end of the lines represent the error factors.

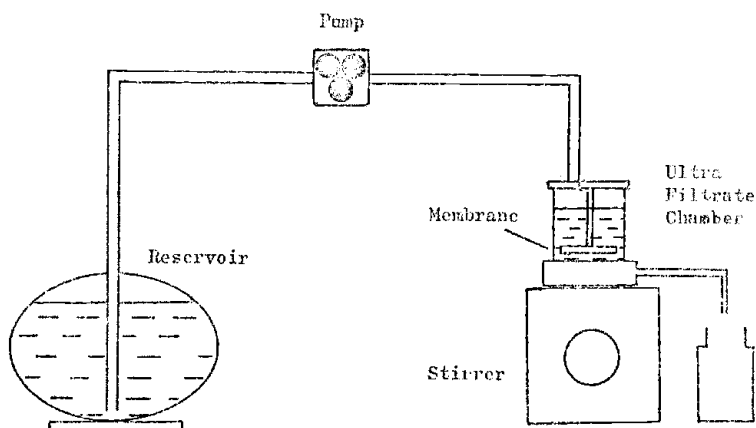


Fig. 14 Diafiltration system.

$$\begin{aligned}
 \text{Moles into chamber} &= \underline{v}_i(\underline{R}) \\
 \text{Moles out of chamber} &= \underline{v}_i(\underline{F})_i \\
 \text{Moles retained} &= \underline{v}_i(\underline{R}) - \underline{v}_i(\underline{F})_i \\
 \text{Total moles } l^{-1} \text{ in chamber} &= \frac{1}{\underline{V}} \sum_{i=1}^n [\underline{v}_i(\underline{R}) - \underline{v}_i(\underline{F})_i]
 \end{aligned}$$

Fig. 15 Calculation of protein binding. \underline{v}_i is the volume of the collected fractions, \underline{R} is the concentration (or radioactive content) of the drug in the reservoir, \underline{F} is the concentration of the drug in the fractions and \underline{V} is the volume in the chamber.

AB). The left hand column values refer to the tissue. In this example, 1 equals plasma, 7 equals adrenals and so on (see also Fig. 12). The remaining values refer to the sample weights combusted and the total sample weights. Figure 12 shows a typical output following the input by the experimenter of the specific activity of the administered radioactive compound and the relevant retention codes (the code 1G would arise from the dpm values). In total there are 44 tissues which can be entered into this program, thus time can be saved and errors in calculations largely overcome.

When metabolite distribution or radiochemical purity work is being carried out, normally by TLC or HPLC, the results are often expressed in the form of histograms. In Fig. 13 an example of this is shown where the percentage distribution values as well as a histogram output are presented. The brackets at the end of the lines represent the error factors.

As a final example, we are often involved in the determination of plasma protein binding. Although we employ several different methods, the one which lends itself to computerized data handling is the Amicon diafiltration method (Fig. 14). Buffer plus the radioactive compound under investigation, contained in the reservoir, is pumped at a constant rate into the ultra-filtrate chamber. The procedure is carried out using either buffer or plasma in the filtration chamber and on each occasion about 50 fractions are generated over a period of approximately 7 h.

These are counted and the retention codes for the dpm values are allotted by the computer. The blank run is needed to allow for any binding of radioactivity to the membrane or to any other part of the equipment. The computer program uses the equations as shown in Fig. 15, where \underline{v}_i is the volume of the collected fractions, \underline{R} is the concentration (or radioactive content) of the drug in the reservoir and \underline{F} is the

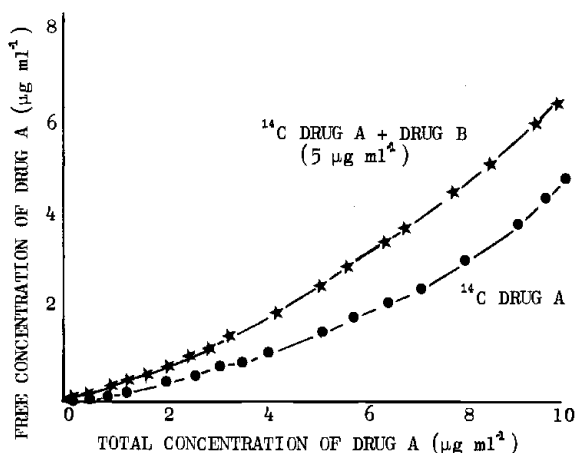


Fig. 16 Protein binding studies.

concentration of the drug in the fractions. The computer needs to know the volume in the chamber, the specific activity of the radioactive compound under investigation and the retention codes for the two sets of dpm values. With this information, the percentage bound and free concentrations are calculated and can then either be plotted manually or using an XY plotter. In Fig. 16 there is a typical example, where the effect of the competitive binder, drug B, on the protein binding of drug A is being examined over a wide concentration range.

CONCLUSIONS

In summary, we opted for an on-line system for liquid scintillation counting. The installation, subsequent testing and ironing-out of teething troubles took about one year. However, we now have a system which we believe is better than off-line methods, where one has the problem of handling and storing punched tape, and most dedicated systems, where there is not the versatility of our procedure or the capability of performing non-dedicated functions. Once the experimenter has a retention code from counting data in which he is confident, then he can use it for further computations as has been demonstrated in this presentation.

REFERENCES

1. P. Johnson, P.A. Rising and T.J. Rising, in Liquid Scintillation Counting, Vol 2 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1972, p.267.

DISCUSSION

R. EVANS: In your dpm output slide you gave an error function column which showed a maximum of the values given of 1.2%. Since this error function was compounded from three error sources, including volume dispensing by micrometer syringe, it seemed that the values given were possibly a little optimistic.

T.J. RISING: The samples counted were all of a high radioactive content and therefore gave low statistical errors in the actual counting. The percent deviation figures quoted by Wellcome for their 'Agla' syringe and the Radiochemical Centre for their standards would not contribute much to this.

D. SMITH: We are currently determining the various pipetting errors involved in certain stages of our work. These will probably be included, where relevant, at a later date.