

## Chapter 23

# Automatic Processing of Data from Liquid Scintillation Counters Illustrated by Drug Distribution Studies

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Liquid scintillation spectrometry is now widely used in biological laboratories for the assay of radioactive isotopes. To facilitate automatic processing of the output from scintillation spectrometers, instruments are available which include small computers in their specifications. Most systems process the data to give only disintegrations/min (d.p.m.) whereas our laboratory takes advantage of a large central computer to process the data in final results form. Computer programs are written in ALGOL and stored in the computer.

In experiments on the distribution of drugs  $^{14}\text{C}$ - and/or  $^3\text{H}$ -labelled drugs are used. The drugs are given to the animal as a rapid intravenous injection at zero time and thereafter blood and urine samples are collected at known time intervals. Samples are analysed by liquid scintillation counting for either drugs or metabolites after appropriate separations. Our data processing technique (see Fig. 1) will be illustrated with reference to the analysis of plasma data in terms of an open two-compartment model of distribution.<sup>1</sup>

The scintillation counter is a small three-channel instrument equipped with a barium-133 external standard, lister and 8-hole paper tape punch. The output consists of sample number, counting time and counts in three channels and is punched simultaneously with the listing. Different experiments are automatically separated on the paper tape so more than one user of the system is possible at any time. The paper tape output is spliced to an identifying lead tape and is then processed to final form by the central computer. The efficiency of counting and d.p.m. of the samples are determined by the 'external standard channels ratio' method.<sup>2</sup> For counting, quenched tritium and carbon-14 standards follow a background bottle. The test samples are placed in the counter after these standards. These latter are made up in a similar manner to the test vials and the number of tritium standards is equal to the number of carbon-14 standards. The degree of quenching of the most quenched tritium standard is equal to that of the similar carbon-14 standard.

Generally samples exhibit a minimum variable degree of quenching and a quadratic function is used to express the relationship between counting efficiency and barium channels ratio of the standards. The coefficients of the quadratic are derived by the method of least squares. Two sets of barium ratios are calculated – for tritium and carbon-14. The highest (maxa) and lowest (mina) ratios of the sets are found and their standard deviation

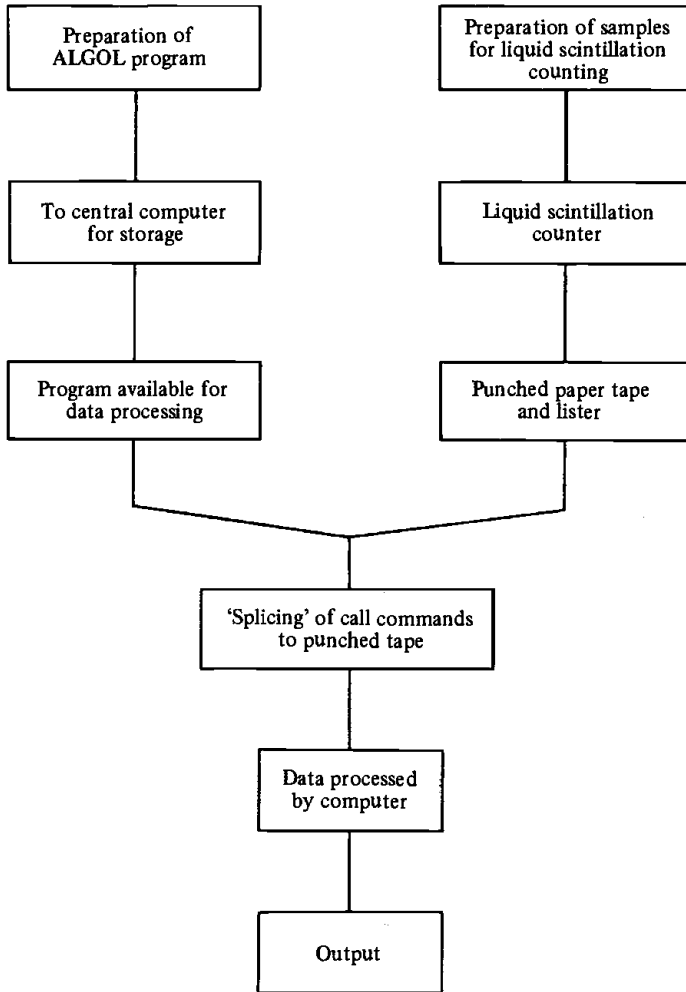


Fig. 1. General procedure.

(SD) calculated. A maximum (max) and minimum (min) allowed ratio is computed as:

$$\text{min} = \text{mina} - (2 \times \text{SD of mina})$$

$$\text{max} = \text{maxa} + (2 \times \text{SD of maxa})$$

If any test ratio is found to be outside the limits of min or max the computer prints a warning message. However, the d.p.m.'s of these vials are still calculated but the extrapolation of the quadratic function is noted. Single or doubly labelled samples are processed by this program and final output includes:

1. the input from the paper tape, which can be compared with the listed output from the scintillation counter as a check for errors in punching or reading of the tape,
2. d.p.m. of the isotopes,

3. SD of tritium samples, and
4. the efficiency of counting of the isotopes in the three channels.

These calculations are performed by the operation of the program SCINTILL, subsequent calculations are worked by other programs in the sequence – DPMTWOCPT which includes SCINTILL. The d.p.m.'s from SCINTILL are used to evaluate concentration terms which are then used as further input for the calculation of the parameters of the two-compartment model of distribution. For the plasma samples, least squares fits are calculated using a biexponential function. The computation is done by the method of residuals<sup>3</sup> and all combinations of biexponential fits to the data are calculated. From these fits the biexponential which has the minimum logarithmic sums of squares of deviations of experimental points to the calculated line is chosen as the biexponential function which best describes the data. The parameters of this function are evaluated in terms of the model – calculations of first order distribution rate constants, fractions of the drug which are located in each compartment with time, etc.

```
begin      declarations

                                     programme body

                                     end
```

Fig. 2. Schematic representation of an ALGOL program.

```
begin      declarations      )
                                     )
                                     ) SCINTILLADDO
                                     )
      programme body      )
```

Fig. 3. Schematic representation of SCINTILLADDO program.

The principle of the data processing technique and the correct 'ordering' of programs is now explained. In Fig. 2 is shown a schematic representation of an ALGOL program. This program has complete block structure<sup>4</sup> and would perform a production sequence. Figure 3 represents a program SCINTILLADDO; this is designed to perform the calculation of d.p.m. from scintillation counting data. However the absence of the final 'end' statement destroys the block structure and this program is not capable of producing output.

In Fig. 4 the program SCINTILL is depicted. The final 'end' is appended to SCINTILLADDO to complete the block arrangement and the running of the program SCINTILL with appropriate data causes the relevant calculations to be executed as pre-

```

SUBSTITUTE SCINTILLADDO  )
                          )
                          ) SCINTILL
                          )
end                    )
    
```

Fig. 4. Schematic representation of SCINTILL program.

DPMTWOCPT:

SUBSTITUTE SCINTILLADDO

SUBSTITUTE ARRAY

SUBSTITUTE BITWO

[ Any minor finishing statements  
that may be required. ]

Fig. 5. Schematic representation of DPMTWOCPT program.

viously described. It is, of course, possible to insert between SCINTILLADDO and 'end' any other program the user may desire providing the rules of block structure in ALGOL are adhered to.<sup>4</sup> This is depicted in Fig. 5 with the program DPMTWOCPT, the working of which was explained. SCINTILLADDO determines d.p.m. as before, ARRAY causes d.p.m.'s to be converted to concentration terms and BITWO the calculations of the biexponential function and parameters of the compartmental model. Minor finishing statements would include those for the completion of block structure, e.g. 'end' to link SCINTILLADDO. The system is flexible and the same programs can be included in different sequences and used for different purposes decided by the user. The simple rules of block structure in ALGOL facilitate this procedure and SCINTILL has been linked to programs which process clearance data from plasma and urine samples.

With this computerized approach to data processing, laboratory results are evaluated in their final form with no manual calculations. The system is simple and can be operated and used with very little training in the techniques of computer programming. Without the aid of the computer, evaluation of results would be time-consuming and laborious with attendant human error.

## REFERENCES

- 1 S. Riegelman, J. C. K. Loo and M. Rowland, *J. Pharm. Sci.* 57, 117 (1968).
- 2 T. C. Hall and C. J. Weiser, *Anal. Biochem.* 17, 294 (1966).
- 3 D. S. Riggs, *The Mathematical Approach to Physiological Problems*, Williams and Wilkins Co., Baltimore, 1963, Chapter 6.

- 4 R. Wooldridge and J. F. Ratcliffe, *An Introduction to Algol Programming*, The English Universities Press Ltd., London, 1963, Chapter 6.

## DISCUSSION

**B. Scales:** It is very interesting to see a program in use for the routine processing of labelled urine and plasma samples with the final output in the form of the required pharmacokinetic constants. However, it must be appreciated that valid results can only be obtained when the radioactivity in the samples is present as a single chemical entity. When more than one labelled chemical species is present, as is often the case due to rapid metabolism even after *intravenous* dosing, then the calculated pharmacokinetic data can be quite meaningless.

**H. E. Barber:** We always take great care and I am sure that these comments do not apply to our results.

**B. Scales:** I am not suggesting that they do but as a general comment, so often people use highly sophisticated techniques and programs, completely ignoring the fact that their initial data are inadequate or inappropriate.

**H. E. Barber:** Yes, we are aware of these problems and check our early and late samples to ensure that only one labelled component is present; otherwise the different labelled components are separated prior to the initial counting procedures.

**P. Johnson:** Comment: Dr. Scales' point has been made at other times during this meeting and cannot be emphasized too strongly.