DATA PROCESSING FOR A MULTI-USER SYSTEM WITH A SMALL ONLINE COMPUTER.

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Abstract. The online processing of the output from liquid scintillation counters with small computers (e.g. 8K memory) raises a series of problems, usually arising from the limitations in program size and in the relatively slow methods of changing programs in store.

A Searle PDS/3 8K computer, both with and without a dual magnetic tape cassette unit, has been used to operate online to several counters. The basic programs derive and store standard curves and apply them as necessary to compute disintegration rates or handle data from radio-immunoassay procedures. User identification is provided, and instructions are given by dialogue programs.

The procedures and times for changing programs are considered; also the required automatic system of checks on the processing procedures and the results, such as graphical displays of standard curves, curve limits, etc. Details of some erroneous results and of limitations imposed by small programs are discussed.

Introduction. The Faculty of Medicine and Dentistry at the University of Birmingham operates a centralised counting service for radioisotopes: over 50 research projects and several postgraduate courses produce samples for liquid scintillation counting. In our experience, the production of samples for counting in most biological and medical work can be termed 'routine', since the sample preparation procedures are well defined for each project and many of the problems have been minimised; thus although care is still needed, the procedures are repetitive and routine. Numerous samples can be rapidly produced and counted automatically on multi-sample machines set for sample or external standard channels ratio determination of quenching. All too often the bottleneck in the procedure occurs at the stage's of data handling and processing, particularly with variably quenched or multi-labelled samples. Consequently, automatic data processors are becoming routine adjuncts to liquid scintillation counting equipment, with the primary objectives of reducing the processing time for the results and the number of man hours expended, and increasing the accuracy and usefulness of the final data by reducing arithmetic errors and utilising more sophisticated processing techniques.

The increasingly common use of small computers (e.g. 8K memory) means that large, complex programs cannot be handled, and small programs have to be interchanged frequently. The fact that many users, with a wide variety of projects, utilise the machines means that programs have to be fairly general in application and flexible, rather than designed for individuals. The provision of a service facility means that most users know little (and do not wish to know more) about the data processing: the final result becomes the only important feature of the process. This makes it imperative to have a comprehensive series of checks of the processing and the data, with an automatic series of visual warnings.

This paper is therefore concerned with practical aspects of data processing as we have experienced and applied them, rather than with advanced aspects of programming, which have been dealt with by many other workers (e.g. 1,2,3).

Materials and Methods. The systems used in these studies were: 1) Philips LSA 00 with 4 counting channels, 2 external standard channels and built-in data analyser. 2) Nuclear Chicago (Searle) Isocap 300 refrigerated counter with PDS/3 8K computer and Teletype. 3) Two Nuclear Chicago (Searle) Isocap 300 refrigerated counters with PDS/3 8K computer, Sykes dual magnetic cassette tape unit and three Teletype outputs.

The programs used were developed by Nuclear Chicago (G.D. Searle, Ltd.) for use in the processing of liquid scintillation results and in radioimmunoassay and competitive protein binding experiments. The programs are designed for multi-user applications, and 12 independent sets of quench correction curves for each counter can be held and recalled automatically.

Results and Discussion. The simplest forms of on-line data analyser used to produce disintegrations per minute (dpm) require the user to predetermine and manually enter the quench correction parameters; the Philips LSA 00 offers this type of data processing. The accuracy is often rather restricted and built-in checks are minimal or missing, but if these limitations are taken into consideration the simple data analysers can be extremely useful.

Computers offer a much more comprehensive system. Easy and rapid access to a large computer is often the best arrangement, since large and sophisticated programmes for a wide range of users can be accommodated, but in many cases this is not feasible and a small computer with limitations in program size and changing has to be utilised.

A computer for use in a multi-user service in a biological or medical environment requires programs, as a basic minimum, for producing dpm and analysing radioimmunoassay results. These programs must handle the results from STANDARD samples to produce, print and store the required parameters of the standard curves, and check the validity and suitability of the curves. The relevant parameters then have to be applied to the results from EXPERIMENTAL samples to produce the required figures (e.g. dpm), after which a basic statistical analysis must be carried out, together with a series of quality control checks. The multi-user application requires a positive IDENTIFICATION to be made (and printed) of the user, the experiment and the set of standard parameters used in the calculation. The instructions to the computer on how to handle each set of standards and experimental samples, and information for identification, are usually given by means of a DIALOGUE. The computer asks a series of questions via the Teletype, and the user types the answers: in a computer with a small core memory, the dialogues may have their own set of programs, to be called up independently of the programs they control.

In a large computer, the programs can all be held in core or on disc and called up rapidly. A major problem with a small computer, particularly handling a variety of results from several counters, is the time taken to change programs. Read-in time for a program occupying 4K of store (e.g. our programs for generating standard curves, or those for handling experimental results) is approximately 15 min using a Teletype paper tape reader, while our radioimmunoassay program takes 25 min. Use of a fast tape reader reduces this time considerably, but manual handling of paper tape is eradicated only by use of a backing store. Our programs are now held on magnetic tape cassettes, and called up via the Teletype keyboard. Call-up time for the dialogues and for dpm programs is 25 to 35 sec, and for the radioimmunoassay system is 55 sec. When the computer is on-line to the counters, it is programmed to call up the requisite programs automatically. The 'dialogue' to give instructions to the computer on how to handle results from a set of samples takes up to one minute for dpm estimations and about 3 minutes for radioimmunoassay procedures, on each set of samples. Three minutes may seem a long time, but the information given (number, replicates and dilutions of standards and unknown, user identification, etc) enables the computer to process completely the data from standard and experimental samples in almost any radioimmunoassay or competitive protein binding assay.

The aspects of time taken in changing programs, and in dialogues, is of particular significance when a small computer is used by many research workers with a variety of problems. A more important problem in the multi-user context is the provision of visual checks on the data and its processing. When one or two people are involved in the development of programs for the computer they rapidly become aware of the limitations of their processing system. Workers in biological or medical fields who use scintillation counters as just one method in their work are usually (not always) relatively uninterested in how a program works and its limitations: as long as a printed result is obtained the user is happy, despite the fact that computers are notoriously lacking in intelligence. The critical ability of the user is often considerably reduced. For these reasons it is even more necessary than usual to have a comprehensive, visual series of checks on processing and the results.

With the possibility of using up to 12 standard curves for each counter, and with many users utilising the counting service, it is important as the first step in eliminating errors to have a printed record of the experimenter, the experiment, and the set of parameters applied to the results: our system uses a fairly standard display as follows:

USER ID. GRESHAM, DIGOXIN. CURVE ID. 3H-SCR

Since many groups of users prepare their own standards, which may then be utilised by other groups, it is necessary to print the basic data for each group of standards, preferably with details of the closeness of the standard curve to each standard point. Thus the range and distribution of the standards is displayed as well as the 'goodness of fit' of the curve. A typical print-out for a set of aldosterone standards in a radioimmunoassay experiment is shown in Table I.

TABLE I. COMPUTER PRINT OUT OF STANDARDS DATA FOR RADIOIMMUNOASSAY EXPERIMENT

ACTUAL DOSE	COMPUTED DOSE	DIFFER-ENCE	PERCENT BOUND
8.0	7.6	0.4	4.68
4.0	4.2	0.2	5.76
2.0	2.1	0.1	7.40
1.0	0.8	0.2	10.72
0.5	0.6	0.1	12.44

The computed curve must be displayed graphically: this is demonstrated clearly by the computed quench correction curve shown in Figure 1, for a set of ^{14}C standards; the curve passes very close to each standard, yet is obviously invalid. An interesting point is that these standards were purchased from a large manufacturer of scintillation counters. The distribution of quenched standards made by our users is usually very much better (but is occasionally much worse!). Another point of interest is that a fairly generally accepted 'rule-of-thumb' is that the power, n, of the fitted curve should not exceed one-half the number of standards, and the curve in Figure 1 obeys this rule: it is a 4th power (quartic) curve and there are 9 standards. It is obvious that rules-of-thumb are potentially dangerous.

This type of procedure is important in all computerised systems - it is indispensible in a multiuser system. The time taken for a print out of these results derived from a set of standards using a Teletype is only 3 to 5 minutes.

However, the print-out of a standard curve can lead to a false sense of security, particularly if a line is strongly curved: an example is shown in Fig 2 where the curve as printed out (Fig 2a) gives an impression of a good fit. Inclusion of the B-zero value, and reprogramming of the computer to align the points in a less strongly curved array, showed the poorness of fit at low concentrations (Fig 2b). A strongly curving or particularly steep or shallow line can mask numerous inaccuracies, particularly in radioimmunoassay procedures where points near the curve limits often have a relatively low level of accuracy. In general, the printed curve should be as nearly rectilinear as possible, and of gradient fairly close to +1 or -1. This can usually be programmed.

The use of a computer makes it a simple process to do a basic statistical analysis of the data: thus when dpm are being calculated, a figure for the best attainable level of accuracy is given. The Nuclear Chicago system prints

DPM	Standard Deviation (absolute) (%)

This gives the accuracy of counting assuming perfect standards, curve fitting, counter and samples. In other words, it gives the level of accuracy that <u>cannot</u> be

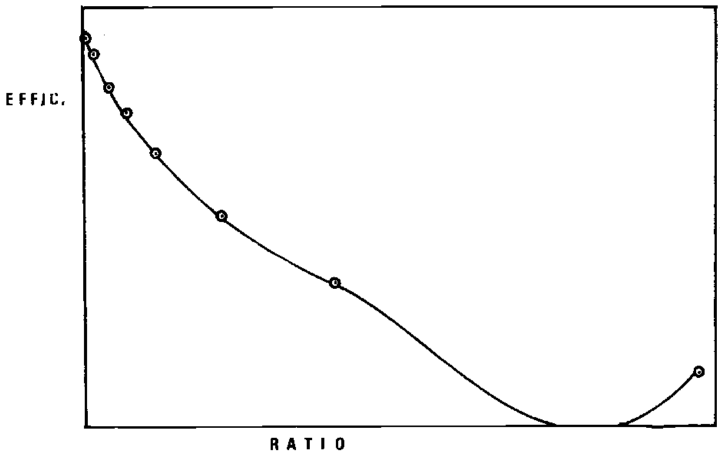

Figure 1. ^{14}C quench correction curve as derived by computer program.

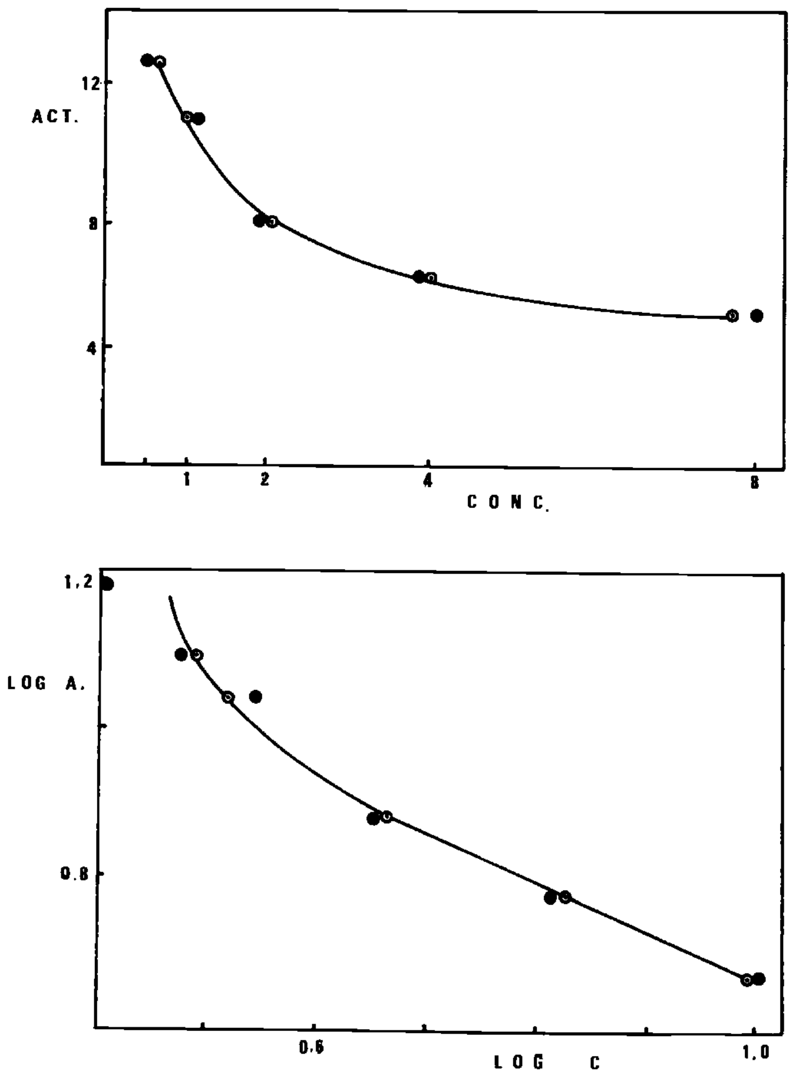

Figure 2. Digoxin radioimmunoassay standard curve. (2a) Linear axes of digoxin concentration and bound activity. (2b) Logarithmic transformation with B_0 value included.

bettered without counting for a longer period. It shows the limit of accuracy but is often taken as representing actual accuracy. Needless to say, the true level of accuracy is usually many times lower.

When the results are printed, a warning must be displayed if the experimental samples fall outside the range of the standards: i.e. if the limits of the quench correction curve are exceeded. The warning printed is:

RESULTS ARE EXTRAPOLATED

We have experienced some highly unusual results when the samples fall outside the range of the standards: in one case we had a group of tritiated samples which the computer calculated as having a counting efficiency of between 700% and 3000%! At that time, no warning messages were printed and a certain amount of confusion ensued. Equally serious are the cases where efficiency of counting is being estimated by the sample channels ratio technique, and some low activity samples are included. The poor statistics of counting can lead to low estimates of counting efficiency, resulting in a higher (and, at first sight, reasonable) estimate of dpm. For this reason it is important to print out channels ratio <u>and</u> efficiency figures.

The use of slightly larger computers allows checks to be made for the presence of luminescence (4), the validity of the set of quenching parameters applied (5), and correct functioning of the counting system (4,5). Application of these type of programs in a small computer would be extremely useful.

The emphasis on a built-in quality control system in a data processing system for liquid scintillation counting is particularly important in a multi-user system, since the programs have to be fairly flexible and general; and also because an expert in (for example) immunology cannot also be expected to be equally expert in all aspects of sample preparation, scintillation mixtures, figures of merit, computer programming, etc., as well as the finer points of radioimmunoassay, gas-liquid chromatography and all the other procedures in common use. This is one reason for running a centralised counting service.

In addition, when a computer is introduced into a scintillation counting system on a routine basis with programs provided, many users apparently lose their critical abilities when surveying their results. Many research workers in the Medical Faculty had a remarkable facility for scanning columns of printed counts and picking out any erroneous results, due to factors such as chemiluminescence. When a computer is introduced into a system, it becomes a 'magic' black box and printed results are accepted as absolute truth. The oft-repeated warning is ignored, that if rubbish is inserted at one end of a computer, one is liable to obtain rubbish accurate to six-significant figures at the other end.

Overall, it can be seen that the use of a small computer in a liquid scintillation counting system offers almost all the advantages which are given by a large computer, and rather more disadvantages which are mainly caused by restricted program capacity and the consequent need to change programs (often by relatively tedious methods). There are significant advantages in using a computer, particularly in the increased accuracy and power of calculation, and the considerable saving in man-hours spent processing data, but in a multi-user service these advantages are tempered by the need to cater for research workers who are not really interested in the functioning of computers (except in so far as they produce a result) or in scintillation counting (except as a convenient technique). However, in our experience, virtually all the problems originate basically from poor preparation of standard or experimental samples, or experimental design: a computer rarely initiates problems, but merely tends to amplify other problems.

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