

A method for measurement and statistical analysis of large numbers of experimental tracings

A. H. CHRISTIANSON, L. C. DINNEEN, G. W. L. JAMES AND A. C. PERKINS

An accurate and rapid method of digitizing large numbers of experimental tracings is possible using a Pencil Follower (D-Mac). Its use in conjunction with a computer has meant that data, hitherto virtually unmanageable, can be subjected to full statistical analysis. An example of its application is cited.

THE acquisition of large numbers of experimental tracings, as from a multi-channel electronic recorder, presents difficulties in the measurement and analysis of the results. Experimental records can be digitized in one of three ways. (1) Manually, by selecting the required results and measuring with a ruler. (2) Automatically, where all the results obtained are converted by electronic apparatus. (3) Semi-automatically, where the experimenter selects results and then converts by electronic apparatus.

The pencil and ruler method of digitizing experimental tracings is often adequate for small amounts of data, such as those from smoked drums, but with larger quantities this is time-consuming, tedious and prone to error.

Automatic conversion is often unsuitable since some form of selection of the results is often required, to eliminate artifacts and other unwanted responses, and to reduce the amount of data collected to manageable proportions.

To overcome these problems we have adopted the semi-automatic method with the use of a Pencil Follower (D-Mac Ltd., Glasgow). To illustrate the principles involved, the method employed in a particular pharmacological experiment is cited.

APPARATUS

The pencil follower provides a way of digitizing data presented in pictorial form, such as pen traces from a multi-channel recorder. There are three main components: a "reading table," approximately $2\frac{1}{2}$ ft \times $4\frac{1}{2}$ ft; a "pencil" which can take one of a variety of forms, the commonest being a small magnifying glass with cross-hairs in its centre; and an "electronic console" which is approximately $1\frac{1}{2}$ ft \times $1\frac{1}{2}$ ft \times 3 ft high.

The graph or tracing to be digitized is placed on the reading table. When the pencil is placed on the table, its position, in terms of its x,y co-ordinates relative to the table, is displayed on the console. This visual display is in the form of two four digit numbers, giving the co-ordinates of the pencil's position to an accuracy of 0.1 mm. A push button on the pencil, or a foot-switch, permanently records the co-ordinates on paper tape or punched cards, or alternatively these co-ordinates may be type-written, by means of an auxiliary output device. The format of the

From the Department of Pharmacological Research, Parke-Davis & Co., Staines Road, Hounslow, Middlesex.

permanent record may be varied by adjusting a plug-board in the console and may include the observation serial number which is recorded automatically. Any titles or comments on the data may be included by the use of a small keyboard which rests on the table top.

METHOD

With care, the baselines of the experimental tracing can be aligned with those of the reading table, a task which is simplified by drawing permanent guide lines on the table. Measurements can then be read directly on the visual display, or typed automatically.

However, the pencil follower is best used with a computer, whether it be on-site or a bureau machine, which has been programmed to make all the subsequent analysis. When so used there is no need for accurate alignment of the graph. It is easier to take two or three baseline points and programme the computer to transform the co-ordinates (see Appendix).

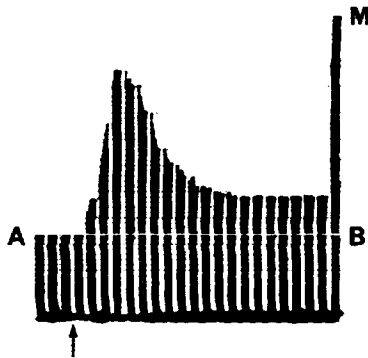


FIG. 1. Intratracheal pressure recording from guinea-pig lung *in vivo*. Guinea-pig, 350 g, anaesthetized with 60 mg/kg i.p. pentobarbitone sodium. Intratracheal pressure was measured on a "Greer" differential micromanometer connected to a Device M8 Polygraph. Recordings were made for 20 sec in each 30 sec. \uparrow , intravenous administration of a bronchoconstricting agent. A, B, baseline x axis; M, maximum possible response.

Fig. 1 shows an experimental tracing in which the intratracheal pressure of a guinea-pig was recorded continuously for 20 sec periods in every 30 sec. Three control periods were recorded to establish a baseline (AB). Recordings were then taken over 10 min, after which the maximum possible response (BM) was obtained.

In a typical experiment, about 12 animals on each of four treatments are used. For each tracing, the baseline points A and B are first plotted by the pencil follower, and then the maximum possible response M, followed by the twenty maximal responses obtained every 30 sec.

Table 1 (A) shows the data output produced by the pencil follower when the tracing in Fig. 1 was digitized. The title, which represents the animal's number and the experimenter's initials, and the asterisks, which terminate the data from a particular animal or treatment, were typed on the keyboard. The output format in this case has been arranged to

A RAPID METHOD OF DIGITIZING TRACINGS

TABLE 1 (A). DATA OUTPUT PRODUCED BY THE PENCIL FOLLOWER IN DIGITIZING FIG. 1. (B) OUTPUT PRODUCED BY THE COMPUTER FROM THE DATA IN TABLE 1A

A			B	
B ₃ GWLJ			B ₃ GWLJ	
*				
0000	235.9	148.1		
0001	289.1	167.9		
0000	275.3	207.1		
**			MAX	41.55
0001	244.1	151.4	1	0.56
0002	244.6	158.8	2	16.83
0003	242.1	172.9	3	50.73
0004	240.5	183.3	4	75.53
0005	243.0	182.6	5	71.85
0006	245.1	182.0	6	68.74
0007	249.1	177.7	7	55.68
0008	253.8	172.3	8	39.56
0009	256.7	170.3	9	32.61
0010	260.7	169.1	10	26.55
0011	262.8	168.0	11	22.30
0012	265.7	168.0	12	19.87
0013	267.8	168.7	13	19.68
0014	270.6	169.1	14	18.24
0015	273.6	170.2	15	18.20
0016	275.2	170.7	16	17.98
0017	276.9	171.4	17	18.14
0018	279.8	172.4	18	17.96
0019	281.6	173.1	19	18.02
0020	284.3	174.1	20	18.01
***			AREA	618.03
			SQRT AREA	24.86

Table 1 (A) comprises an animal identification (B3) and an operator identification (GWLJ). * End of title; **, end of baseline and maximum possible co-ordinates; ***, end of data for animal B3. Left hand column, serial number of operation; middle column, X co-ordinate; right hand column, Y co-ordinate.

In Table 1 (B), the animal and operator identifications (B3 GWLJ) are reprinted. MAX is the absolute value of the maximum possible response. Left hand column, serial number of operation; right hand column, y co-ordinate as a percentage of MAX. AREA is the estimate of the area under the % response curve. SQRT AREA is the square root of the area.

include the automatic counting device, which is reset to zero for each animal before and after the two baseline points are digitized. All the data are punched directly onto paper tape. A print-out of this tape is then obtained from a teleprinter-tape reproducer set and the tape can be corrected if necessary before being sent to a computer.

Table 1 (B) shows the results produced by the computer from the data in Table 1 (A). The animal title is printed at the top followed by the absolute value of the maximum response and then the twenty readings, which are expressed as percentages of the maximum possible response (M). For each treatment the mean % response is also calculated. In addition, the area under each % response curve is estimated by means of the Trapezium rule. An analysis of Variance model (Davies, 1954) is used to test for differences in mean areas between treatments. To obtain minimal residual variance, the areas are transformed before analysis to square root areas (Bartlett, 1947).

DISCUSSION

We have found that laboratory technicians learn to use the system readily, basic instruction taking less than one day. Complete analysis of an experiment of the type illustrated can be made in about 2 hr, compared with the two or three days it took with pencil and ruler methods.

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The cost of the computer analysis is more than offset by this saving in technicians' time, since they are freed from much tedious and repetitive work. The system has also proved less prone to error, since the technicians do not, at any stage, have to write down any results or make any calculations.

APPENDIX: TRANSFORMATION OF CO-ORDINATES

If X, Y represent the co-ordinate axes of the reading table and x, y those of the tracing to be digitized, then if two baseline points on the tracing are taken (Fig. 2).

- (1) A (X_1, Y_1) at the origin of the graph,
- (2) B (X_2, Y_2) at any other point on the graph's x-axis,

then the co-ordinates of any point C (X_3, Y_3) plotted on the table, with respect to the origin of the graph are:

$$x = \frac{(X_3 - X_1)(X_2 - X_1) + (Y_3 - Y_1)(Y_2 - Y_1)}{\sqrt{(X_2 - X_1)^2 + (Y_2 - Y_1)^2}}$$

and
$$y = \frac{(Y_3 - Y_1)(X_2 - X_1) - (X_3 - X_1)(Y_2 - Y_1)}{\sqrt{(X_2 - X_1)^2 + (Y_2 - Y_1)^2}}$$

In the particular example cited, we were not directly concerned with the x co-ordinate and therefore A was placed at any convenient point on the x-axis of the graph.

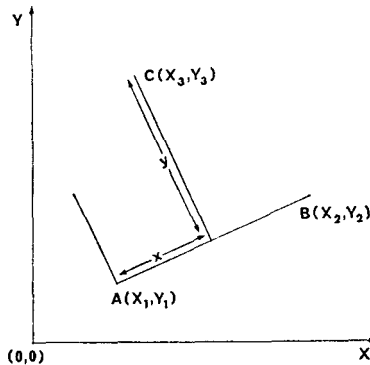


FIG. 2. The transformation of the co-ordinates of a point, C, from those of the reading table to those of the trace. X, Y , the co-ordinate axes of the reading table. AB, the baseline of the trace. x, y , the co-ordinates of the point C relative to the trace.

References

Bartlett, M. S. (1947). *Biometrics*, 3, 39-52.
 Davies, O. L. (1954). *Statistical Methods in Research and Production*, 2nd edn, pp. 71-117, London: Oliver & Boyd.