MICROCOMPUTER PROGRAMS FOR QUANTITATIVE AUTORADIOGRAPHY AND STEREOLOGY

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ABSTRACT

4 BASIC programs for quantitative autoradiography and stereology were written for a Hewlett-Packard HP 85 microcomputer. Program "PILOTE" is to choose the main parameters for a combined autoradiographic and stereological study. Program "ITEMS" is to compute data for the "circle method". Program "HISTO" is to build histograms of distances between silver grains and membranes. Program "SOURCE-ITEM" is to compute data for the "hypothetical grain distribution" method. These 4 programs were used to analyse the ultrastructural localization of membrane receptors using 125-I labelled ligands in cultured nerve cells.

INTRODUCTION

Microcomputers, now widely used in biology, are powerful tools for the quantitative analysis of biological structures.

BASIC programs were written for a HP-85 Hewlett-Packard microcomputer to speed up the analysis of data for stereologic and quantitative autoradiographic studies of neuronal cell cultures. The purpose of this investigation was to localize surface receptors and to determine receptor density on mouse N 115 neuroblastome cells and on other neuronal systems in vitro or in vivo. The surface receptors studied were voltage-sensitive sodium channels which bind specifically a α-scorpion toxin (α-ScTx) Couraud et al. 1982).

MATERIAL AND METHODS

Mouse neuroblastoma cells were cultivated as previously described (Couraud et al. 1982). Differentiated cells were incubated with 125 I-labelled α-ScTx (10^{-9} M) or a mixture of labelled (10^{-9} M) and native (10^{-7} M) α-ScTx.

Following standard EM processing, autoradiograms were prepared using the "flat-substrate" method (Salpeter and Bachmann 1972). Subsequently a stereological analysis and a quantitative autoradiographic study were carried out on the sections in order to calculate cell surface area, and to calculate the number of silver grains and their localization. BASIC programs were written for a HP 85 microcomputer (32 kilobytes, advanced programming and matrix ROMs, digitizer, plotter and printer).

RESULTS

Program "PILOTE"

This program was used to choose the main parameters of a combined autoradiographic and stereological study from data collected from a small sample :

- number of micrographs and type of grid for point and intersection counting to calculate the volume density (V_v) and/or surface density (S_v) as described by Weibel (1979),

- number of micrographs and of circles to be applied for the "circle method" of Williams (1969),

- background density estimate from sections of embedding medium without tissue as described by Williams (1978).

This program plotted out the final composite grid (points, lines, circles) used in the main experiment. The grid was drawn onto transparent overlay used in a 35 mm film back projector (Weibel 1979).

Program "ITEMS"

This was used to compute data for the "circle method" (Williams 1969). Input for each cell compartment (item) was : number of silver grains, number of circles, number of points and/or intercepts. The program performed the chi-square test between the grain and circle distributions and calculated, for each item, the crude specific relative radio-

activity, V_v and/or S_v, and grain per unit V_v or S_v. Finally the density of binding sites was calculated according to the formula of Fertuck and Salpeter (1976).

Program "HISTO"

This was used to compute data for the "grain density" method (Salpeter et al. 1969). From measured distances between silver grains and a line source, the program plotted out a cumulative histogram and calculated the HD value of our experimental conditions. The HD value is then used to plot histogram of distances between grains and membranes of neuroblastoma cells.

Program "SOURCE-ITEM"

This was written to compute data obtained by the following methods : the "hypothetical grain distribution" method (Blackett and Parry 1973), the "mask" method (Salpeter et al. 1978). This program improved the analysis of "junctional" items such as cell membranes and associated cell microvilli.

For each micrograph, the cell membrane perimeter was digitized and stored in file. The program plotted out a transparent overlay with hypothetical sources localized on the cell surfaces and hypothetical grains produced by disintegration at these hypothetical sources. Directions were randomly chosen as were distances between hypothetical sources and grains from the "universal curve" published by Salpeter et al. (1974) which was stored in file. Sources were distributed at random or systematically on cell surfaces.

From the observed grain distribution and the matrix of hypothetical grain/source distribution, the program computed the source matrix giving the hypothetical grain distribution closest to the observed grain distribution, using a multilinear regression method. Source density was then calculated.

APPLICATIONS

In neuroblastome cell cultures, α-ScTx binding sites were localized on cell membranes and their density estimated. For all cell plasma membranes, the site density is 1.016 ± 0.49 per square micron of surface (Mean $\pm$ SD). The calculated distance is about 992 nm between 2 supposedly randomly

distributed sites.

DISCUSSION AND CONCLUSION

Quantitative analysis of biological structures was speeded up by using a microcomputer and specialized programs. Individual data from each micrograph of a sample were stored in file and submitted to subsequent calculations. Obtention of the final results was very easy and the programs allowed a good reproductibility of all the calculations by decreasing "bugs" coming from hand-made data collection and calculations.

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