COMPUTER ANALYSIS FOR DATA FROM COMPETITIVE PROTEIN-BINDING ASSAYS

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A versatile computer program which provides rapid analysis of data from all types of competitive protein-binding assay methods was developed. The method computes bound/free ratios or percent binding, prints the standard curve, and permits the interpolation of unknown values over the entire range of standard curve values. The present algorism is adaptable to any available computer or programmable calculator with plotting capability.

Radioimmunoassay (RIA) and other competitive protein-binding assay methods have revolutionized diagnostic endocrinology. Furthermore, the use of this assay methodology has provided a clearer understanding of basic endocrine physiology. However, each assay generates a tremendous volume of raw data. The manual calculation of bound to free (B/F) ratios, standard curves, and the interpolation of hormone concentrations is not only time-consuming, but also permits the introduction of considerable error.

Recently, several computer programs have been designed to compute, analyze, and summarize RIA data (1, 2). However, in many academic, hospital, and institutional settings the sophisticated hardware necessary for these methods is not readily available. Therefore, the algorism presented here represents a rapid, dependable, and extremely adaptable method for analyzing data from presently available competitive protein-binding assays, which can be processed by any computer or programmable calculator with the ability to manipulate arrays of numerical values and plot output data. The number of samples which can be processed will depend upon the available memory of the system used.

MATERIALS AND METHODS

This computer program was originally designed to process data from a standard calcitonin RIA which measures the competitive binding of ^{131}iodine-labeled porcine calcitonin and endogenous calcitonin by a guinea pig antiporcine calcitonin serum. A detailed description of this assay method has previously been reported (3).

A flow diagram of the assay data analysis method is presented in Figures 1a and 1b. The flow diagram of the plotting routine used in this technique is illustrated in Figure 2. The assay data analysis method can readily be converted to calculation of percent binding rather than B/F ratios and used with the plotting routine outlined in Figure 2. The output presented in this communication was created by a FORTRAN IV program and processed on an IBM System/360.

RESULTS AND DISCUSSION

A typical standard curve derived by this algorism is illustrated in Figure 3. The standard curve was created by plotting the B/F ratios computed from the measured bound and free values of the samples of known hormone concentration, i.e., standard B/F ratios, against the corresponding known hormone concentrations, i.e., hormone standards. A slight discrepancy in the shape of the computer-derived curve may arise because of the printer inability to place points between axially designated values. This printer artifact does not affect the accuracy of hormone interpolation since the actual computed standard curve values are used for the interpolation of hormone concentrations.

Several previously reported RIA computer programs have employed curve-fitting procedures which restrict hormonal calculations to specific areas of the standard curve (4, 5). The present program permits utilization of the entire range of standard curve values for hormonal interpolation. A typical calcitonin standard curve employed a 600-fold working range of standard hormone concentration values

(0.025 to 15 ng). Furthermore, since the standard curve is linearly fitted between standard hormonal values, the program can be used for analysis of any competitive protein-binding assay data. However, calculation accuracy requires closely placed standard values in nonlinear areas of the standard curve. A comparison between the data derived from this computer method and the same data processed manually is presented in Table 1. The mean difference ($\bar{D}$) between the two sets of data was -0.008 and the standard error of the difference ($S_{\bar{D}}$) was 0.018 (6). The small mean difference ($\bar{D}$) and difference variation ($S_{\bar{D}}$), together with the observation that the largest difference between the sets of data (± 0.08) is within the routine experimental

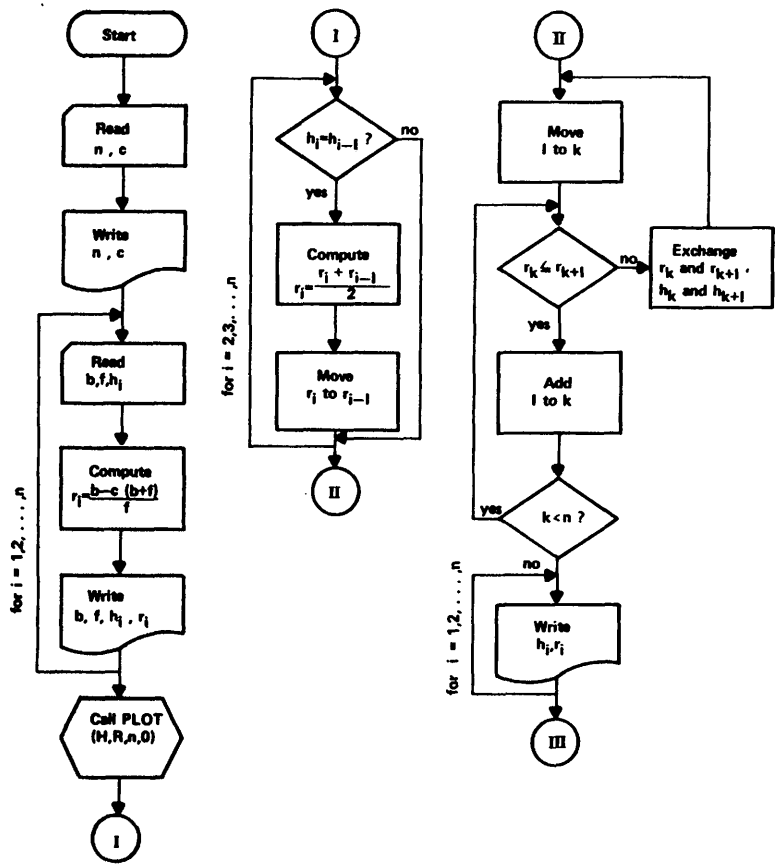


FIGURE 1a. Flow diagram of the assay data analysis method. For samples of known hormone concentration:

n = number of samples
c = correction factor
b = bound cpm for a particular sample
f = free cpm for a particular sample
H = (h_1, h_2 . . . , h_n): set of hormone concentrations
R = (r_1, r_2 . . . , r_n): set of bound/free ratios

error for this method, indicate that the computer program is a reliable method for assay data analysis.

The program also plots the unknown B/F ratios against the interpolated hormone concentration (Fig. 4). This plot illustrates the working areas of the standard curve used for hormone interpolation. Since the greatest accuracy is provided by the center of the standard curve, this plot indicates whether changes in the volume of unknown should be made in subsequent assays.

Data readout provides a record of bound and free CPM, B/F ratio, interpolated hor-

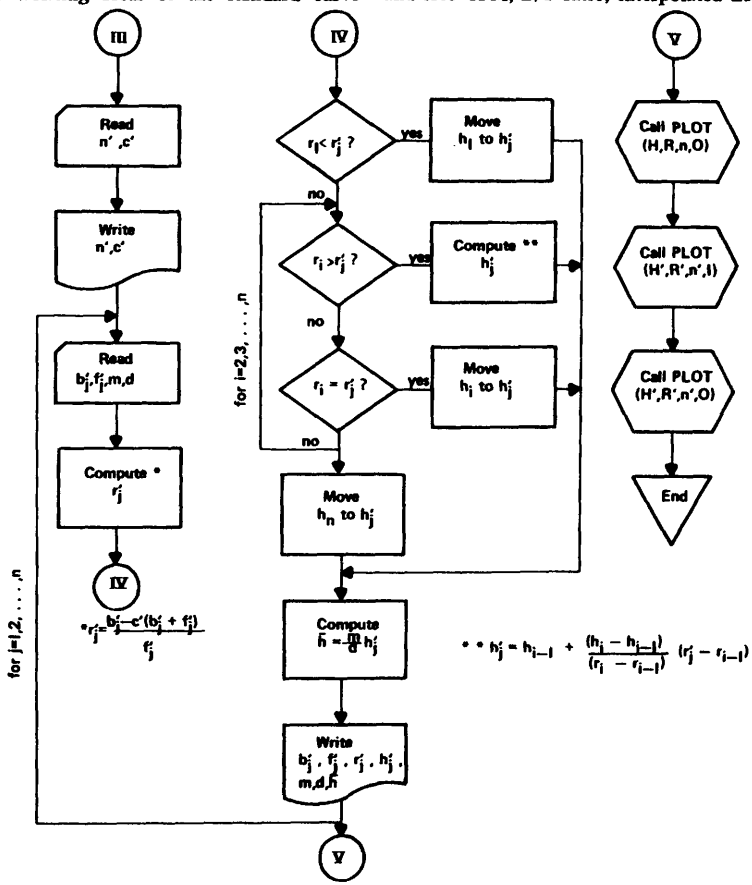
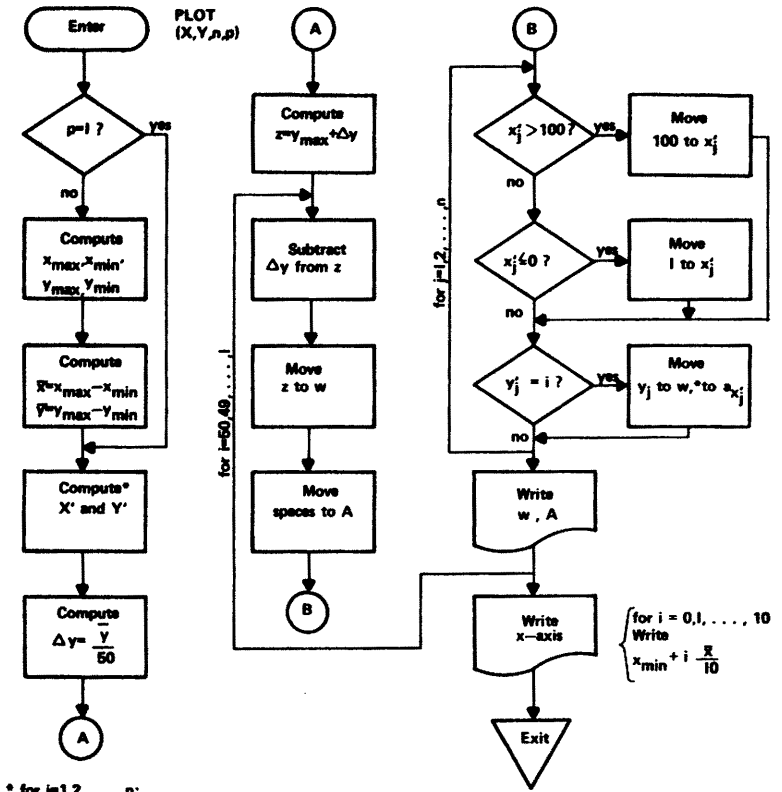


FIGURE 1b. Flow diagram of the assay data analysis method (continued). For samples of unknown hormone concentration:

n' = number of samples
c' = correction factor
B' = (b'1, b'2, . . ., b'): set of bound cpms
F' = (f'1, f'2, . . . f'): set of free cpms
m = multiplication factor for a particular sample
d = division factor for a particular sample
H' = (h'1, h'2, . . ., h'n): set of interpolated hormone concentrations
R' = (r'1, r'2, . . ., r'n): set of bound/free ratios



* for i=1,2, . . . ,n;

$$x_j' = \left[\frac{99(x_j - x_{min})}{\bar{x}} + 1.5\right]$$

$$y_j' = \left[\frac{49(y_j - y_{min})}{\bar{y}} + 1.5\right]$$

where [z] denotes the integral portion of a real number z.

FIGURE 2. Flow diagram of the plotting routine.

n = number of points to be plotted

$X = (x_1, x_2, \ldots, x_n)$: set of x-coordinates at points to be plotted

$Y = (y_1, y_2, \ldots, y_n)$: set of y-coordinates at points to be plotted

p = value of 1 indicates that points will be plotted within the range of the previous curve plotted

x_{min}, x_{max}: smallest and largest elements of X

y_{min}, y_{max}: smallest and largest elements of Y

$X' = (x'_1, x'_2, \ldots, x'^n)$: set of scaled x-coordinates

$Y' = (y'_1, y'_2, \ldots, y'^n)$: set of scaled y-coordinates

$A = (a_1, a_2, \ldots, a_{100})$: 100 position line used for plotting

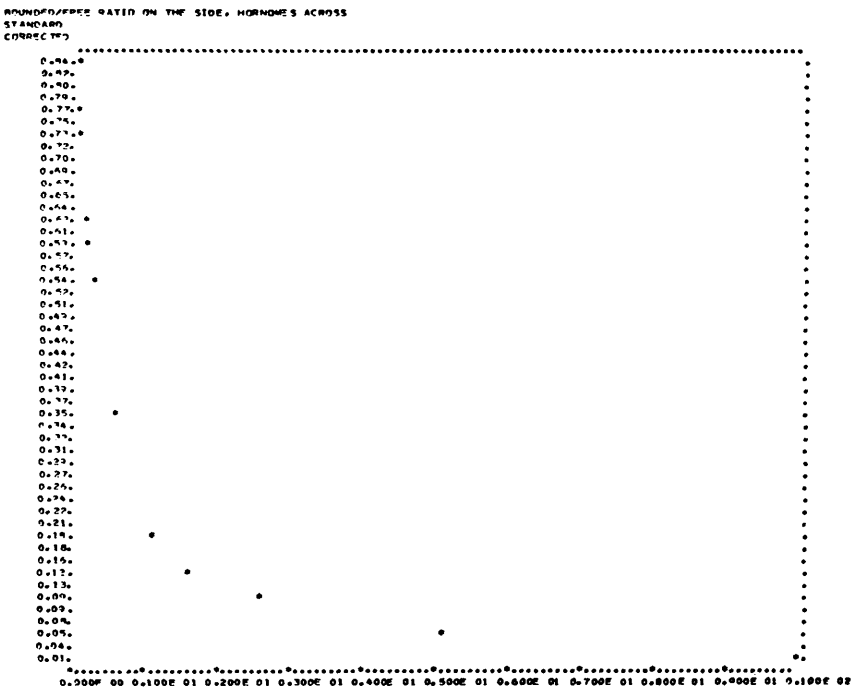


FIGURE 3. Computer derived standard curve. The standard B/F ratios are on the ordinate and hormone standards on the abscissa.

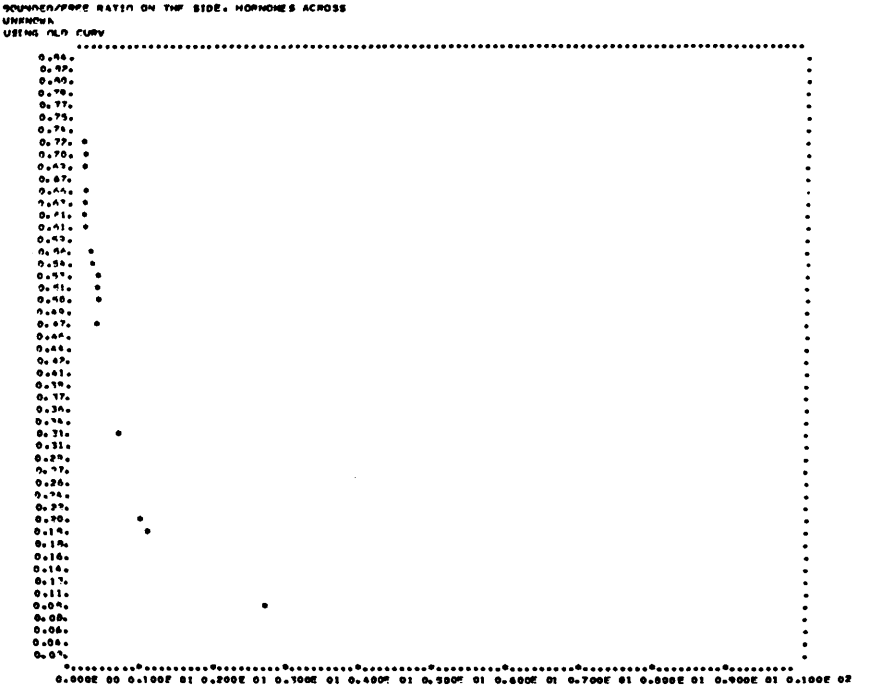


FIGURE 4. Computer plot of unknown B/F ratios versus interpolated hormone concentrations. The unknown B/F ratios are on the ordinate and interpolated hormone concentrations on the abscissa.

mone concentration, multiplication factor (MF), division factor (DF) and corrected hormone conentration (interpolated hormone concentration × MF ÷ DF). The multiplication factor and division factor can be used to process the interpolated hormone data into any convenient form (i.e. ng hormone/ml, ng hormone/mg tissue, etc.).

TABLE 1. *Comparison of manual and computer calculated assay data.*

Number	Calcitonin Concentration (ng/ml) Manual calculation of interpolated hormone concentrations	Computer calculation of interpolated hormone concentrations	Difference
1	8.85	8.93	+0.08
2	6.22	6.29	+0.07
3	7.00	7.03	+0.03
4	5.25	5.17	−0.08
5	7.57	7.51	−0.06
6	6.15	6.09	−0.06
7	7.95	7.95	0.00
8	5.45	5.44	−0.01
9	8.25	8.33	+0.08
10	0.67	0.67	0.00
11	0.88	0.80	−0.08
12	0.90	0.83	−0.07
			$\bar{D} = -0.008$
			$S_{\bar{D}} = 0.018$

This computer program was designed to process radioimmunoassay data; however, because of its flexible design, the program can also be used for other competive-binding assays, such as the radioreceptor assay, immunoradiometric assay, and plasma-ligand assay. Furthermore, any presently used separation method should be applicable.

Copies of the computer program are available from the first author (J. T. P.), Department of Pharmacology, College of Pharmacy, Norman, Oklahoma 73069.

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