Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 77–94. Pergamon Press. Printed in Great Britain

A SIMPLE COMPUTER PROGRAM FOR QUANTITATION AND SCATCHARD ANALYSIS OF STEROID RECEPTOR PROTEINS

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(*Received* 28 *May* 1976)

SUMMARY

This paper presents a relatively simple program for the analysis of data in tissue receptor protein assays. Information is presented to the computer as c.p.m. obtained from liquid scintillation systems. The program is adaptable to clinical studies while retaining sufficient flexibility to deal with cross-competition data and the generation of regression lines for Scatchard plot analyses.

INTRODUCTION

Increasing interest in the correlation between steroid receptor protein content and the response of tissue to hormonal manipulation has necessitated a system for rapid data analysis. The treatment of breast cancer and leukemia are areas in which the characterization of tissue in terms of steroid receptor activity is of increasing clinical significance [1–7]. Unfortunately, however, the computations involved in receptor analysis are tedious, prone to error, and are particularly laborious if one desires to generate a Scatchard plot with any degree of statistical confidence [8, 9].

The majority of computer programs currently available are designed to linearize conventional radioimmunoassay data [10, 11]. In standard radioimmunoassays a single level of radioligand is utilized and levels of non-labeled material are varied to generate a standard curve based on displacement of radioligand from a fixed quantity of binding material. The level of non-specific binding is therefore constant throughout the assay and the assay component of interest in unknown samples is the amount of non-radioactive material present and capable of displacing radioactivity. In contrast, analysis of tissue for receptor protein presupposes an unknown but fixed amount of tissue preparation and varies the quantity of radioligand to which the preparation is exposed. The information derived from such a procedure, when analyzed by a variety of techniques including Scatchard analysis, permits determination of the affinity of the preparation for the compound of interest and the number of bonding sites available to the compound. In many cases however, and particularly in clinical studies where only limited quantities of tissue are available, it is not feasible to generate a Scatchard plot by the above procedure and the examination of a tissue preparation is limited to binding capacity at a single level of steroid thought sufficient to saturate most tissue receptor sites. Additionally, in clinical studies a number of different steroids may well be of interest, technical procedures may vary with the steroid and tissue under investigation and consequently computations on a single tissue sample may be complex. A program dealing with sucrose density gradient isolation of 4 and 8 *S* steroid binding components is available [12]. However a program which facilitates rapid data analysis with minimum restrictions on methodology, permitting almost any competitive binding assay as well as Scatchard analysis, would be of considerable interest.

The program presented here utilizes BASIC, a computer language closely allied and readily translatable to FORTRAN and FOCAL. The programming commands are relatively simple, consisting of reserving memory space for variables and sets of variables and of operative commands for the mathematical manipulation of these variables. More complex commands related to the particular capacities of the 9830A calculator and to format presentation have either not been introduced or could readily be deleted from the program without affecting the process of data reduction. Segmentation of the program into additional files other than the three employed in this case would also permit data processing by comparable systems lower in memory capacity than the 1760 words which limit the 9830A. In contrast the program can readily be extended if more complex computer systems are in fact available.

The system is designed to apply a least squares fit of data to a simple regression analysis. It will not accurately describe a function which is curvilinear and is thus too simplified for a strict analysis of binding in which cooperativity exists or in which two classes of binding sites are known to exist. The graphical presentation does however immediately indicate to even an inexperienced operator that such a situation may exist. The program is specifically designed for input of data for a Scatchard plot

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although manipulation of the variables to generate a classic binding curve or a Lineweaver–Burke plot rather than a Scatchard plot is feasible. However it is not suitable for radio-immunoassay binding data although it can be used to transform data from classic dissociation experiments in which the amount of competing cold steroid is varied against a constant amount of radioactive ligand.

The present program does require a more sophisticated printer than is standard for most small programmable desktop calculators. The format is designed for clarity of presentation to those unfamiliar with receptor analysis and has proven extremely simple and time saving, allowing the operator to examine a variety of Scatchard plots through options for data selection. Data reduction is extremely rapid.

EXPERIMENTAL

A Hewlett–Packard model 9830A desktop calculator (Hewlett–Packard Calculator Products Division, Loveland, CO) programmed in BASIC was used for this program. The counting system employed was a Packard TriCarb model 544 linked to a teletype (Teletype Corp., Skokie, OH) for printout and optional punch tape generation. The computer utilizes cassette tapes divided into files, greatly simplifying program and data transferral into memory. The statistical procedures applied to data transformation were taken from *Statistical Methods* by Snedecor and Cochran[13].

The details of procedures for analyses of tissue for receptor binding activity have been previously described [1, 14–16]. Aliquots of tissue cytosol are incubated with known concentrations of radioactive ligands in the presence and absence of much higher concentrations of non-radioactive steroids or analogs. The difference between non-competed incubation tubes and the competed incubation tubes is considered to represent specifically bound radioligand. It is possible however to generate non-specific binding incubation tubes by alternative procedures such as employing competitors or inhibitors of specific binding, i.e. nafoxidine [17]. The program also does not proscribe the use of sucrose density gradient, charcoal, or protamine sulfate precipitation in the separation of bound and free radioligand and consequently becomes adaptable to most methods of establishing non-specific binding and most types of tissue preparations including whole cell as well as cytosol or nuclear fractions. In addition the program is not restricted to steroid receptor protein analysis but can deal with any compound or hormone of interest.

The lead into the program permits identification of the assay as the operator desires and the selection of either a program for receptor analysis or a conventional RIA program. Initial manual data input includes the specific activity of the radioligand in question, efficiency of the liquid scintillation system, incubation volume of the assay, volume of assay material utilized, and information on the number and molarities of radioligand involved in the assay. Program loops permit a scan of a variety of receptor classes at a large number of concentrations.

Subsequent data input involves the total counts observed to be present in the incubation at each molarity of radioligand. The computer then presents a printout of the predicted versus experimentally derived molarities used for each incubation. The experimental data will be used in later data transformations. Entry of the counts observed in non-competed incubations results in a printout of the average totally bound c.p.m. and mol/l. This average value will later be used in the computation of specifically bound radioligand, thus avoiding the necessity for equal numbers of non-competed and competed incubations tubes. The computer produces a summary output of data on entry of the c.p.m. for competed incubation tubes. An average specifically bound value and its 95% confidence limits are printed in terms of fmol bound/ml of cytosol and the operator may select an optional print of fmol/unit protein or whatever final units are desired. This feature is particularly convenient for clinical studies and occurs at each concentration of radioligand employed. Additionally, provision has been made at various points in the program to correct errors made in data entry and for the selection or deletion of information at the operator's discretion. This aspect lends considerable flexibility to the system and allows errors to be corrected without rerunning the entire program.

At the conclusion of data entry on a given radioligand the operator has an option to proceed to enter new data or to conduct regression analysis on the set of data in computer memory. The B (specifically bound fmol/ml of cytosol) and B/F values are printed for each incubation sample (in effect for each competed incubation tube) and for the average of incubation samples at a given concentration of radio-ligand prior to election of a Scatchard analysis. An inspection of these values permits at least a rough estimate of the feasibility of this procedure. If a Scatchard is in fact constructed the operator may edit data freely either before or after drawing the initial Scatchard plot. The subsequent format includes a printout of the coefficients for the line, the K_D, and N value (presented in the units desired by the operator, i.e. fmol/mg protein), and the regression coefficient. The plot itself is then presented graphically. The operator can edit the plot as frequently as desired.

RESULTS

The program (Table 3) itself with an explanatory commentary (Table 1) and a list of variables (Table 2) is presented below. This is followed by a typical printout of data obtained from a clinical sample (Table 4).

Table 1. Program commentary

Line	Commentary
	File 1
10–100	The operator may input any information desired concerning the assay.
110–210	A branch to either receptor or RIA programs.
	File 2
10–90	Any additional identification of the assay.
100–150	Record the sample weight and the total vol. of cytosol obtained from the sample. If this information is irrelevant input 1.
160–190	Input a decimal (i.e. 0.408) for the counting efficiency of the vials used to establish the total amount of radioligand added to incubation tubes. It is assumed that these vials are of uniform quench.
200–250	Enter decimals indicating the incubation vol. and the vol. of cytosol used per tube.
260–360	The operator may indicate the terms in which binding capacity is to be described. A choice of per unit protein [18] or per cell is indicated here but any other units can readily be substituted. If a value is unknown or the information is irrelevant input 1.
370–410	A loop permitting a scan of the binding of up to 8 different radioligands.
420–450	Enter the name of the radioligand.
460–500	Enter the specific activity of the radioligand in Ci/mol.
510–530	Enter a decimal for the efficiency of the counting system with respect to incubated samples. Again quench is assumed to be uniform among any given set of samples for a single radioligand.
540–560	Input as a decimal the fraction of the incubated samples which has been utilized for counting. For example an aliquot from a dextran coated charcoal assay (0.8) may be counted whereas the entire sample (1.0) is counted in protamine sulfate assays.
600–620	Enter the number of different concentrations of radioligand used to investigate the binding of that radioligand. This loop permits up to 15 different molarities to be analyzed.
630–640	Input the molar concentration of radioligand predicted to be present in a given set of incubation tubes and the number of counting vials used to establish total counts added to the incubation. It is most convenient to enter the predicted molarities utilizing a floating point notation. Thus 2 counting vials at 5×10^{-9} M can be expressed as 5E-09,2. It is mandatory to input the highest concentration first.
650–820	Entry of the c.p.m. observed in the total counts vials results in a printout of the experimental *vs.* the predicted molarities used in the incubation. The experimental figure will be used in any subsequent computations.
830–850	The operator may correct any errors in data entry by entering 1, thus returning program execution to line 630.
860–880	Enter the number of replicates for non-competed and competed incubation tubes; i.e. 2, 2.
890–1060	Enter the c.p.m. observed in non-competed tubes. Each number must be entered with an execute command.
1070–1290	Entry of the c.p.m. observed in each competed tube will result in a printout of the mean fmol bound/ml of cytosol.
1300–1370	The operator may again indicate the final units in which binding capacity is to expressed.
1380–1400	Any information desired concerning a particular set of incubation samples can be entered; the nature of the competitor, incubation temperature, etc.
1410–1450	Indication that an error has been made in data input (1) will return the program to line 870 permitting re-entry of information. No error (0) will cause the program to loop and information on the next lowest concentration of radioligand will be requested beginning with line 630. This loop will continue until the lowest concentration of a particular radioligand has been entered.
1460–1600	The computer presents a printout of the Bound and the Bound/Free ratios for each individual point and for the average B and B/F values at each concentration of radioligand. Note each point has been obtained by subtracting data for an individual competed incubation tube from the average of non-competed tubes at the corresponding concentration of radioligand.
1610–1670	Entering 1 causes the computer to load File 3 of the tape cassette for processing of a Scatchard plot. It is advisable to manually scan the B and B/F values to see whether a Scatchard analysis is in fact feasible since once the program has proceeded to file 3 it will be necessary to reload file 2 to process another radioligand. Entering 0, on the other hand, transfers program execution to line 410 and information on the next radioligand is requested. If there is no additional radioligand the program ends. It is necessary to point out that limitations on computer memory make it impossible to retain information on more than one radioligand and the last set of data entered is consequently the only set in computer memory and the only set which can be processed for a Scatchard plot.
	File 3
10–200	The operator may choose to use average B and B/F values for each molarity of radioligand by entering 1 or to use all points by entering 0.
210–220	If the lowest concentrations of radioligand are not useful Scatchard values the operator may instruct the computer to ignore those values by inputting the last sample number to be used in regression analysis.
230–490	The operator may correct or delete any individual values. Entering 0 indicates no further modifications are necessary.
500–560	A printout of the data which will be used for regression analysis is produced.

Table 1 (continued)

Line	Commentary
570–990	The coefficients of the regression line are generated. A value for N, the number of binding sites, is presented with an operator option for units per mg protein or sites per 10^6 cells. The K_D is also described as is the regression coefficient. If the data does not permit a Scatchard analysis, due to a negative x-intercept. ERROR will appear on the display. Entering RUN, execute will restart the program in file 3 and the operator may edit data as desired.
1000–1310	A graph of the Scatchard is presented. In this process the computer will attempt to renumber samples in order of increasing B/F value and the y-intercept is treated as a sample. If values are highly erratic the printout will reflect this.
1320–1330	The operator may edit the graph by entering 1. 0 ends the program.
1340–1410	A printout of the sample numbers and values as they are currently in computer memory is presented. These are the numbers to be considered in further editing.
1420–1430	The program will loop to line 230 or end.
1440–1610	A subroutine to renumber samples in order of increasing B/F values.

Table 2. List of variables

E1	Efficiency (total counts)
E3	Incubation volume
E8	Volume cytosol per incubation tube
E9	Units protein (cell count, etc.) per ml cytosol
N4	Number of different radioligands in assay (1–8)
E2	S.A. of radioligand
E5	Efficiency (incubation samples)
N1	Number of different concentrations of a given radioligand (1–15)
T(K, I)	TS(8, 15)—Predicted molarity for up to 15 concentrations of 8 different radioligands
L(I)	LI(15)—Number of replicates, total count vials (1–4)
S(I, J)	SS(15, 4)—c.p.m. observed in total counts vials for up to four replicates at 15 concentrations of radioligand
U(I)	US(15)—average experimental molarity of up to 15 different concentrations of radioligand
R	Formula for 95% confidence limits
L(I)	LI(15)—Number of replicates, uncompeted incubation samples (1–4)
C(I)	CI(15)—Number of replicates, competed incubation samples (1–4)
B(I, J)	BS(15, 4)—c.p.m., uncompeted incubation tubes, for up to 4 replicates at 15 concentrations of radioligand
W(I)	WS(15)—average mol/l. of radioligand bound in non-competed incubation tubes at up to 15 different concentrations of radioligand
D(I, J)	DS(15, 4)—c.p.m., competed incubation tubes, for up to 4 replicates at 15 different concentrations of radioligand
X(I, J)	X(15, 4)—moles of radioligand specifically bound per incubation tube (later transformed to fmol/ml of cytosol) for up to 4 replicates at 15 concentrations of radioligand)
F(I, J)	FS(15, 4)—free moles of radioligand per incubation tube
Y(I, J)	Y(15, 4)—B/F ratio for up to 4 replicates at 15 concentrations of radioligand
Z(I)	ZS(15)—average specifically bound fmol/ml cytosol at up to 15 different concentrations of radioligand
M(I)	MS(15)—Average B/F ratio at up to 15 concentrations of radioligand
N2	Number of points used for Scatchard analysis
W(N2)	Values of B as used for a Scatchard analysis
T(N2)	Values of B/F as used for a Scatchard analysis
S1–S5	Sum of squares for regression line
B1–B8	Coefficients for the regression line

TABLE 3

PROGRAM LISTING

FILE I

```
10 DIM A$[25],B$[10],D$[20]
20 DISP "ASSAY NAME";
30 INPUT A$
40 DISP "ASSAY DATE";
50 INPUT B$
60 DISP "TECHNOLOGIST";
70 INPUT D$
80 PRINT
90 PRINT A$;"                    "B$;"            "D$;
100 PRINT
110 DISP "RECEPTOR ASSAY (1) OR RIA (2)";
120 INPUT H1
130 IF H1=2 THEN 180
140 DISP "THIS IS FOR RECEPTOR STUDIES!"
150 WAIT 3000
160 LOAD 2
170 END
180 DISP "THIS IS A RADIOIMMUNOASSAY "
190 WAIT 3000
200 LOAD 5
210 END
```

FILE II

```
10 COM X[15,4],Y[15,4],N1,E3,E8,E9,CI[15],MS[15],ZS[15]
20 DIM TS[8,15],FS[15,4],SS[15,4],BS[15,4],DS[15,4],US[15],WS[15]
30 DIM A$[30],LI[15]
40 DISP "NAME OF PATIENT";
50 INPUT A$
60 PRINT A$
70 PRINT "-----------------------------------------------------------------"
80 PRINT
90 PRINT
100 DISP "TISSUE WEIGHT (GRAMS)";
110 INPUT E9
120 PRINT "TISSUE WEIGHT (GRAMS) ="E9
130 DISP "VOLUME OF SAMPLE CYTOSOL (ML)";
140 INPUT E9
150 PRINT "TOTAL VOLUME OF SAMPLE CYTOSOL (ML) ="E9
160 DISP "EFFICIENCY RATIO,TOTALS";
170 INPUT E1
180 FIXED 4
190 PRINT "EFFICIENCY ="E1
200 DISP "INCUBATION VOLUME (L)";
210 INPUT E3
220 PRINT "INCUBATION VOLUME IN LITERS ="E3
230 DISP "VOL. CYTOSOL (LITERS) ASSAYED  ";
240 INPUT E8
250 PRINT "VOL. CYTOSOL = "E8"L."
260 DISP "WHOLE CELL=1; PROTEIN=0";
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```
270 INPUT H1
280 IF H1#0 THEN 340
290 DISP "LOWRY (MG/ML) =; (IF UNKNOWN =1";
300 INPUT E9
310 IF E9=1 THEN 370
320 PRINT "LOWRY (MG/ML)="E9
330 GOTO 370
340 DISP "CELL COUNT/ML =; (IF UNKNOWN =1)";
350 INPUT E9
360 PRINT "CELL COUNT/ML ="E9
370 DISP "# OF DIFFERENT HOT STEROIDS USED";
380 INPUT N4
390 FOR K=1 TO N4
400 FIXED 0
410 DISP "STEROID #"K" =";
420 INPUT A$
430 PRINT
440 PRINT "                    "A$
450 PRINT
460 DISP "SPECIFIC ACTIVITY (CURIES/M)";
470 INPUT E2
480 PRINT "SPECIFIC ACTIVITY OF STANDARD (CURIES/M) = "E2
490 E4=E1*E2*E3*(2.2E+12)
500 FIXED 4
510 DISP "EFFICIENCY RATIO FOR SAMPLES ";
520 INPUT E5
530 PRINT "EFFICIENCY(SAMPLES) ="E5
540 DISP "FRACTION OF SAMPLE FOR COUNTING";
550 INPUT E6
560 PRINT "FRACTION FOR COUNTING ="E6
570 E7=E2*E3*E5*E6*(2.2E+12)
580 PRINT
590 FIXED 1
600 DISP "NUMBER OF LEVELS (I) OF STANDARD ";
610 INPUT N1
620 FOR I=1 TO N1
630 DISP "CONC.(M/L), REPLICATES/LEVEL"I"";
640 INPUT T[K,I],L[I]
650 DISP "CPM FOR TOTAL TUBES?";
660 V=Q=0
670 FOR J=1 TO L[I]
680 INPUT S[I,J]
690 PRINT S[I,J];
700 V=V+S[I,J]/E4
710 Q=Q+(S[I,J]/E4)*(S[I,J]/E4)
720 NEXT J
730 IF L[I]<2 THEN 750
740 R=2*SQR(ABS((Q-V↑2/L[I])/(L[I]-1)))
750 PRINT "MEAN TOTAL CPM = "V*E4/L[I]
760 U[I]=V/(L[I])
770 FLOAT 3
780 IF L[I]<2 THEN 810
790 PRINT "PREDICTED M/L ="T[K,I]" EXPERIMENTAL M/L="U[I]" +/- "R
800 GOTO 820
810 PRINT "PREDICTED M/L ="T[K,I]" EXPERIMENTAL M/L ="U[I]
820 PRINT
830 DISP "IF ERROR TYPE 1, IF NOT TYPE 0 ";
840 INPUT H1
850 IF H1=1 THEN 630
860 FIXED 1
870 DISP " # BOUND, # NSB TUBES AT LEVEL"I"";
880 INPUT L[I],C[I]
890 DISP "CPM FOR BOUND TUBES AT LEVEL"I"";
900 V=Q=0
910 FOR J=1 TO L[I]
920 INPUT B[I,J]
930 PRINT B[I,J];
940 V=V+B[I,J]/E7
950 Q=Q+(B[I,J]/E7)*(B[I,J]/E7)
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```
960 NEXT J
970 PRINT "MEAN CPM = "V*E7/L[I];
980 IF L[I]<2 THEN 1000
990 R=2*SQR(ABS((Q-V↑2/L[I])/(L[I]-1)))
1000 W[I]=V/L[I]
1010 FLOAT 3
1020 IF L[I]<2 THEN 1050
1030 PRINT "MEAN BOUND M/L="W[I]"+/-"R
1040 GOTO 1060
1050 PRINT W[I]
1060 PRINT
1070 DISP "CPM FOR COMPETING TUBES/LEVEL"I"";
1080 V=Q=O=U=0
1090 FOR J=1 TO C[I]
1100 INPUT D[I,J]
1110 O=O+D[I,J]
1120 D[I,J]=D[I,J]/E7
1130 X[I,J]=(W[I])-(D[I,J])
1140 F[I,J]=(U[I])-(X[I,J])
1150 Y[I,J]=X[I,J]/F[I,J]
1160 U=U+Y[I,J]
1170 V=V+X[I,J]
1180 Q=Q+X[I,J]*X[I,J]
1190 FIXED 1
1200 PRINT D[I,J]*E7
1210 NEXT J
1220 PRINT "MEAN NSB CPM ="O/C[I];
1230 Z[I]=V/C[I]*(1E+12)
1240 M[I]=U/C[I]
1250 IF C[I]<2 THEN 1290
1260 R=2*SQR(ABS((Q-V↑2/C[I])/(C[I]-1)))
1270 PRINT "MEAN BD.(FM/ML INCUBATION)="Z[I]"+/-"R*(1E+12)
1280 GOTO 1300
1290 PRINT "FM BOUND/ML INCUBATION ="Z[I]
1300 IF E9=1 THEN 1380
1310 DISP "WHOLE CELL BINDING=1; PROTEIN=0";
1320 INPUT H1
1330 IF H1#0 THEN 1360
1340 PRINT "MEAN BD. (FM/MG PROTEIN) ="(Z[I]/E9)*E3/E8
1350 GOTO 1380
1360 PRINT "MEAN BOUND (SITES/CELL)="6E+08*(Z[I]/E9)*E3/E8
1370 GOTO 1380
1380 DISP "IDENTIFICATION";
1390 INPUT A$
1400 PRINT A$
1410 DISP "ERRORS? INPUT 1 IF YES; 0 IF NO";
1420 INPUT H1
1430 IF H1=1 THEN 870
1440 PRINT "-----------------------------------------"
1450 NEXT I
1460 PRINT
1470 N2=0
1480 PRINT "          N                FM BOUND/ML INC.                B/F"
1490 PRINT
1500 FOR I=1 TO N1
1510 FOR J=1 TO C[I]
1520 FLOAT 3
1530 X[I,J]=X[I,J]*(1E+12)
1540 N2=N2+1
1550 FIXED 6
1560 PRINT "     "N2;"          "X[I,J]"                    "Y[I,J]
1570 NEXT J
1580 PRINT
1590 PRINT "MEAN X ="Z[I];"MEAN Y ="M[I]
1600 PRINT
1610 NEXT I
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```
1620 DISP "SCRATCHARD=1;NO SCATCHARD=0";
1630 INPUT H1
1640 IF H1#1 THEN 1670
1650 LOAD 3
1660 END
1670 NEXT K
1680 END
```

FILE III

```
10 COM X[15,4],Y[15,4],N1,E3,E8,E9,C[15],MS[15],ZS[15]
20 DIM T$[30],W$[30],B[10]
30 FIXED 3
40 N2=0
50 DISP "SCATCHARD BASED ON AVERAGES = 1";
60 INPUT H1
70 IF H1#1 THEN 140
80 FOR I=1 TO N1
90 N2=N2+1
100 W[N2]=Z[I]
110 T[N2]=M[I]
120 NEXT I
130 GOTO 210
140 FOR I=1 TO N1
150 FOR J=1 TO C[I]
160 N2=N2+1
170 W[N2]=X[I,J]
180 T[N2]=Y[I,J]
190 NEXT J
200 NEXT I
210 DISP "# OF POINTS FOR SCATCHARD";
220 INPUT N2
230 DISP "CORRECTIONS=1;DELETIONS=2;NONE=0";
240 INPUT H1
250 IF H1=0 THEN 500
260 IF H1=2 THEN 380
270 DISP "CORRECTION IN SAMPLE #";
280 INPUT N3
290 FIXED 0
300 DISP "X("N3")=";
310 FIXED 5
320 INPUT W[N3]
330 FIXED 0
340 DISP "Y("N3")=";
350 FIXED 5
360 INPUT T[N3]
370 GOTO 230
380 DISP "DELETE SAMPLE #";
390 INPUT I
400 N3=I
410 W[N2+1]=W[N3]
420 T[N2+1]=T[N3]
430 FOR N3=I TO N2
440 W[N3]=W[N3+1]
450 T[N3]=T[N3+1]
460 NEXT N3
470 DISP "# OF SAMPLES NOW = ";
480 INPUT N2
490 GOTO 230
500 FIXED 6
```

```
510 PRINT "            N              FM BOUND/ML INC.              B/F    "
520 PRINT
530 FOR N3=1 TO N2
540 PRINT "       "N3;"        "W[N3];"         "T[N3]
550 NEXT N3
560 PRINT
570 FLOAT 5
580 S1=S2=S3=S4=S5=0
590 FOR N3=1 TO N2
600 S1=S1+W[N3]
610 S2=S2+W[N3]*W[N3]
620 S3=S3+T[N3]
630 S4=S4+(T[N3]*T[N3])
640 S5=S5+W[N3]*T[N3]
650 NEXT N3
660 PRINT
670 PRINT
680 PRINT "THE EQUATION FOR A STRAIGHT LINE IS:  Y = A + BX"
690 PRINT
700 B1=(S5-((S1*S3)/N2))/(S2-((S1)↑2)/N2)
710 B2=(S3/N2)-(B1*S1/N2)
720 B3=SQR(((S4-((S3)↑2/N2))-(S5-(S1*S3/N2)))/(N2-2))
730 B4=SQR(ABS((S4-((S3)↑2/N2))/(N2-1)))
740 B5=SQR((S2-((S1)↑2/N2))/(N2-1))
750 B6=B3/SQR(S2-((S1)↑2/N2))
760 B7=(S5-S1*S3/N2)/(N2-1)/B5/B4
770 B8=-B2/B1
780 PRINT "B = SLOPE OF REGRESSION LINE ="B1
790 PRINT
800 PRINT "K(D) ="(-1/B1)/(1E+12)"MOLES/L"
810 PRINT
820 FIXED 5
830 PRINT "A = THE Y INTERCEPT OF THE LINE ="B2
840 PRINT
850 PRINT "-A/B = THE X INTERCEPT OF THE LINE ="B8" FM/ML"
860 PRINT
870 DISP "WHOLE CELL BINDING=1; PROTEIN=0";
880 INPUT H1
890 IF H1=1 THEN 940
900 PRINT "THE NUMBER OF BINDING SITES, N ="B8" FM/ML INCUBATION VOLUME OR"
910 PRINT (B8/E9)*E3/E8" FM/MG PROTEIN OR "B8*(6.023E+08)*E3/(E8*E9)" SITES/MG"
920 PRINT
930 GOTO 970
940 PRINT "THE # OF BINDING SITES, N ="B8" FM/ML OR "B8*E3/(E9*E8)"FM/10↑6 CELL"
950 PRINT "OR "B8*(6.023E+08)*E3/(E8*E9)" SITES PER 10↑6 CELL"
960 PRINT
970 PRINT "R = THE REGRESSION COEFFICIENT = "B7
980 PRINT
990 PRINT
1000 PRINT
1010 FIXED 5
1020 L=N2+1
1030 K=52/B8
1040 T[L]=B2
1050 W[L]=0
1060 IF T[L]<T[L-1] THEN 1130
1070 IF T[L-1]>T[L-2] THEN 1100
1080 GOSUB 1540
1090 GOTO 1060
1100 J=30/T[L]
1110 PRINT T[L];TAB12+K*W[L];"X"W[L]
1120 GOTO 1200
1130 GOSUB 1450
1140 GOTO 1070
1150 IF T[L]>T[L-1] THEN 1170
1160 GOSUB 1450
1170 IF L-1=1 THEN 1200
1180 IF T[L-1]>T[L-2] THEN 1200
1190 GOSUB 1540
1200 FOR I=1 TO (T[L]-T[L-1])*J
1210 PRINT TAB12;"!"
1220 NEXT I
1230 PRINT T[L-1];TAB12+K*W[L-1];"X"W[L-1]
1240 L=L-1
1250 IF L#1 THEN 1150
1260 FOR I=1 TO T[L]*J
```

```
1270 PRINT TAB12;"!"
1280 NEXT I
1290 PRINT TAB10;"0 !----------
1300 PRINT TAB12+K*B8;"X"
1310 PRINT TAB9+B8*K;B8
1320 PRINT
1330 DISP "TO MODIFY GRAPH =1; NO CHANGE=0";
1340 INPUT H1
1350 PRINT
1360 PRINT "          N        FM BOUND/ML        B/F"
1370 PRINT
1380 FIXED 6
1390 FOR N3=1 TO N2
1400 PRINT "      "N3;"        "W[N3];"         "T[N3]
1410 NEXT N3
1420 PRINT
1430 IF H1=1 THEN 230
1440 END
1450 E1=T[L]
1460 E2=T[L-1]
1470 T[L]=E2
1480 T[L-1]=E1
1490 E1=W[L]
1500 E2=W[L-1]
1510 W[L]=E2
1520 W[L-1]=E1
1530 RETURN
1540 E1=T[L-2]
1550 E2=T[L-1]
1560 T[L-1]=E1
1570 T[L-2]=E2
1580 E1=W[L-1]
1590 E2=W[L-2]
1600 W[L-1]=E2
1610 W[L-2]=E1
1620 RETURN
1630 END
```

TABLE IV

?PROGRAM EXECUTION

CLINICAL 2/1/76 M G.
SMITH,MARY CH. OF D'EAST

TISSUE WEIGHT (GRAMS) = 0.571
TOTAL VOLUME OF SAMPLE CYTOSOL (ML) = 1
EFFICIENCY = 0.4020
INCUBATION VOLUME (4 LITER) = 0.0002
VOL. CYTOSOL = .1 0.01 L.
LOWRY (MG/ML)= 2.8260

E2 - CHARCOAL ASSAY

SPECIFIC ACTIVITY OF STANDARD (CUNTS/ML) = 91300
EFFICIENCY(SAMPLES) = .3390
FRACTION FOR COUNTING = 0.6670

```
 32141.0
 32426.0    MEAN TOTAL CPM =  32283.5
PREDICTED M/L = 2.500E-09   EXPERIMENTAL M/L= 1.999E-09   +/-  2.496E-11

 269.0
 279.0
 297.0   MEAN CPM =  281.7   MEAN BOUND M/L= 3.101E-11  +/- 3.124E-12

 235.0
 238.0
 236.0
MEAN NSB CPM = 236.3   MEAN BD.(FM/ML INCUBATION)= 5.0  +/- 0.3
MEAN BD. (FM/MG PROTEIN) = 3.5
0
----------------------------------------

 17105.0
 19001.0    MEAN TOTAL CPM =  18053.0
PREDICTED M/L = 1.250E-09   EXPERIMENTAL M/L= 1.118E-09   +/-  1.660E-10

 160.0
 150.0
 153.0   MEAN CPM =  154.3   MEAN BOUND M/L= 1.699E-11  +/- 1.130E-12
 123.0
 145.0
 136.0
MEAN NSB CPM = 134.7   MEAN BD.(FM/ML INCUBATION)= 2.2  +/- 2.4
MEAN BD. (FM/MG PROTEIN) = 1.5
0
----------------------------------------

 7756.0
 7826.0  MEAN TOTAL CPM =  7791.0
PREDICTED M/L = 6.250E-10   EXPERIMENTAL M/L= 4.824E-10   +/-  6.130E-12

 76.0
 74.0    MEAN CPM =  75.0    MEAN BOUND M/L= 8.257E-12  +/- 3.114E-13

 55.0
 58.0
 63.0
MEAN NSB CPM = 58.7    MEAN BD.(FM/ML INCUBATION)= 1.8  +/- 0.9
MEAN BD. (FM/MG PROTEIN) = 1.3
0
----------------------------------------

        N              FM BOUND/ML INC.              B/F

     1.000000            5.137600                 0.002577
     2.000000            4.807300                 0.002411
     3.000000            5.027500                 0.002521

MEAN X = 4.990800   MEAN Y = 0.002503

     4.000000            3.449500                 0.003095
     5.000000            2.128400                 0.001908
     6.000000            3.119200                 0.002798

MEAN X = 2.899030   MEAN Y = 0.002600

     7.000000            2.201810                 0.004585
     8.000000            1.871540                 0.003894
     9.000000            1.321080                 0.002746

MEAN X = 1.798140   MEAN Y = 0.003742

          N             FM BOUND/ML INC.             B/F

       1.000000           4.990800            0.002503
       2.000000           3.449500            0.003095
       3.000000           1.798140            0.003742
```

```
THE EQUATION FOR A STRAIGHT LINE IS:  Y = A + BX

B = SLOPE OF REGRESSION LINE =-3.88076E-04

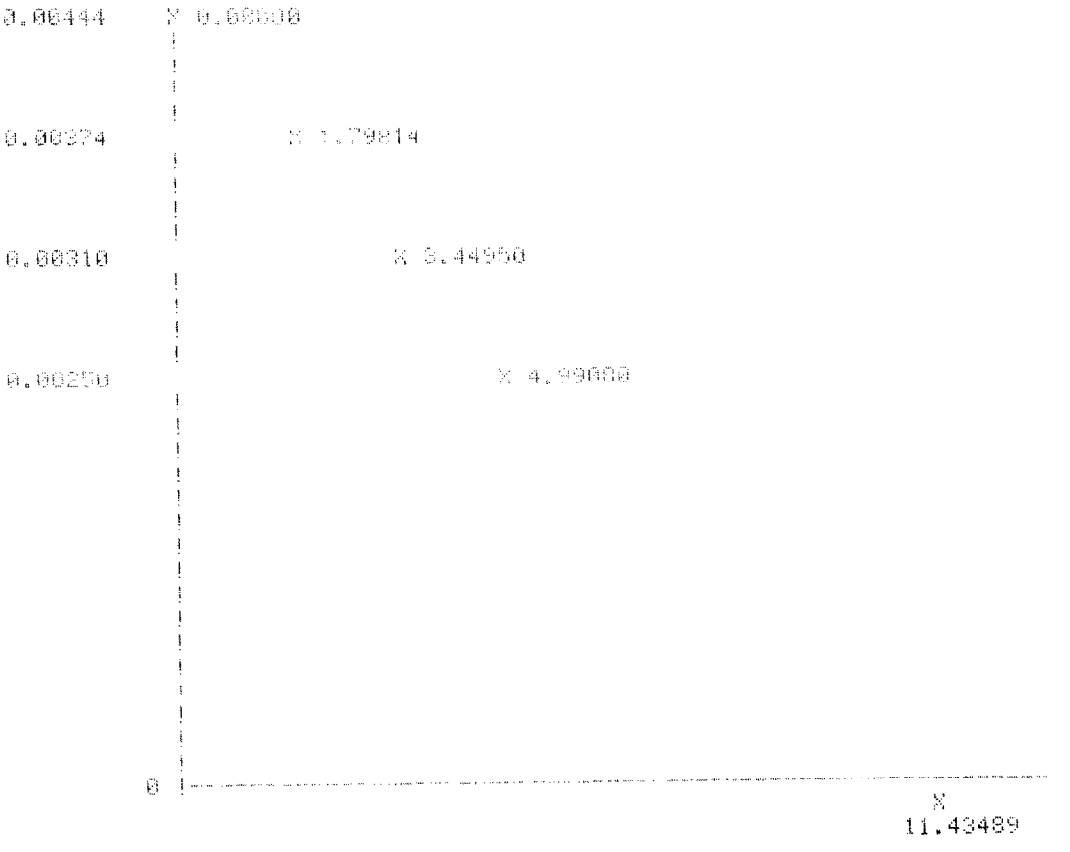
K(D) = 2.57681E-09   MOLES/L

A = THE Y INTERCEPT OF THE LINE = 0.00444

-A/B = THE X INTERCEPT OF THE LINE = 11.43489    FM/ML

THE NUMBER OF BINDING SITES, N = 11.43489    FM/ML INCUBATION VOLUME OR
 8.09264     FM/MG PROTEIN OR  4874194397.20000     SITES/MG

R = THE REGRESSION COEFFICIENT = -0.99998
```



DHT - PROTAMINE SO4

```
SPECIFIC ACTIVITY OF STANDARD (CURIES/M) =  130000.000000
EFFICIENCY(SAMPLES) = 0.4180
FRACTION FOR COUNTING = 1.0000

 180606.0
 178092.0   MEAN TOTAL CPM =  179349.0
PREDICTED M/L = 1.000E-08   EXPERIMENTAL M/L= 7.800E-09   +/-  1.546E-10

 3719.0
 3645.0
 3461.0  MEAN CPM =  3608.3  MEAN BOUND M/L= 1.509E-10  +/- 1.111E-11

 3210.0
 3227.0
 3296.0
MEAN NSB CPM = 3244.3  MEAN BD.(FM/ML INCUBATION)= 15.2    +/- 3.9
MEAN BD. (FM/MG PROTEIN) = 10.8
0
```

```
 87680.0
 85951.0     MEAN TOTAL CPM =  86815.5
PREDICTED M/L = 5.000E-09   EXPERIMENTAL M/L= 3.776E-09   +/-  1.063E-10

 1960.0
 1812.0
 1743.0  MEAN CPM =  1838.3  MEAN BOUND M/L= 7.689E-11  +/- 9.274E-12

 1622.0
 1576.0
 1658.0
MEAN NSB CPM = 1618.7  MEAN BD.(FM/ML INCUBATION)= 9.2  +/- 3.4
MEAN BD. (FM/MG PROTEIN) = 6.5
0
----------------------------------------

 38875.0
 39101.0     MEAN TOTAL CPM =  38988.0
PREDICTED M/L = 2.500E-09   EXPERIMENTAL M/L= 1.696E-09   +/-  1.390E-11

 796.0
 794.0   MEAN CPM =  795.0   MEAN BOUND M/L= 3.325E-11  +/- 1.183E-13

 708.0
 683.0
 661.0
MEAN NSB CPM = 684.0   MEAN BD.(FM/ML INCUBATION)= 4.6  +/- 2.0
MEAN BD. (FM/MG PROTEIN) = 3.3
0
----------------------------------------

        N              FM BOUND/ML INC.              B/F

     1.000000            16.660000                0.002141
     2.000000            15.949000                0.002049
     3.000000            13.063000                0.001678

MEAN X = 15.224000  MEAN Y = 0.001956

     4.000000             9.048000                0.002402
     5.000000            10.971900                0.002915
     6.000000             7.542300                0.002002

MEAN X = 9.187400   MEAN Y = 0.002440

     7.000000             3.638700                0.002151
     8.000000             4.684300                0.002770
     9.000000             5.604500                0.003316

MEAN X = 4.642500   MEAN Y = 0.002746

          N              FM BOUND/ML INC.              B/F

       1.000000            15.224000            0.001956
       2.000000             9.187400            0.002440
       3.000000             4.642500            0.002746


THE EQUATION FOR A STRAIGHT LINE IS:  Y = A + BX

B = SLOPE OF REGRESSION LINE =-7.49577E-05

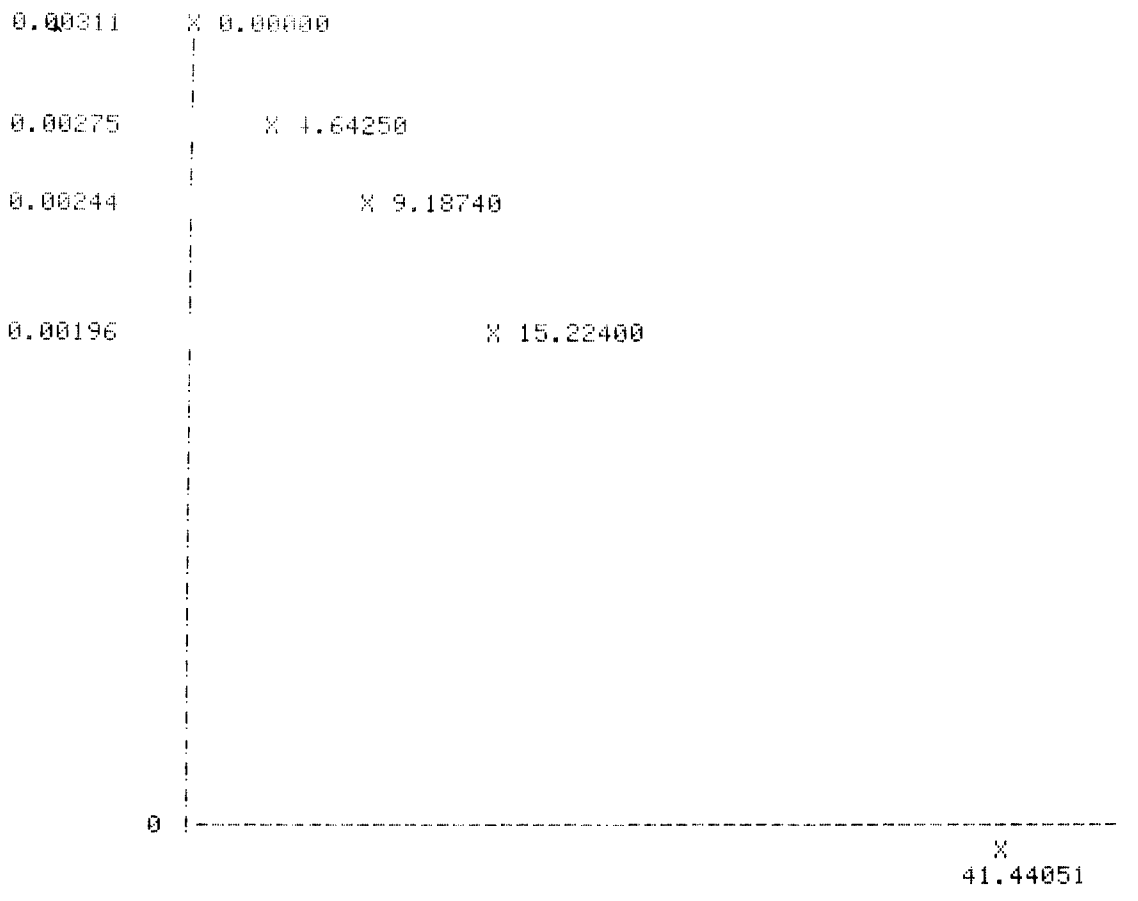
K(D) = 1.33409E-08   MOLES/L

A = THE Y INTERCEPT OF THE LINE = 0.00311

-A/B = THE X INTERCEPT OF THE LINE = 41.44051    FM/ML

THE NUMBER OF BINDING SITES, N = 41.44051    FM/ML INCUBATION VOLUME OR
 29.32803    FM/MG PROTEIN OR  17664272153.20000    SITES/MG

R = THE REGRESSION COEFFICIENT = -0.99886
```



DEX-CHARCOAL ASSAY

SPECIFIC ACTIVITY OF STANDARD (CURIES/M) = 28000
EFFICIENCY(SAMPLES) = 0.3390
FRACTION FOR COUNTING = 0.6670

451801.0
464255.0 MEAN TOTAL CPM = 458028.0
PREDICTED M/L = 1.000E-07 EXPERIMENTAL M/L= 9.248E-08 +/- 3.556E-09

4182.0
4173.0 MEAN CPM = 4177.5 MEAN BOUND M/L= 1.500E-09 +/- 4.569E-12

3866.0
3778.0
MEAN NSB CPM = 3822.0 MEAN BD.(FM/ML INCUBATION)= 127.6 +/- 44.7
MEAN BD. (FM/MG PROTEIN) = 90.3
0

N	FM BOUND/ML INC.	B/F
1.000000	111.820000	0.001211
2.000000	143.410000	0.001553

MEAN X = 127.615000 MEAN Y = 0.001382

```
                PROG + 10-6M F - CHARCOAL

SPECIFIC ACTIVITY OF STANDARD (CURIES/M) =  91400
EFFICIENCY(SAMPLES) = 0.3390
FRACTION FOR COUNTING = 0.6670

 77380.0
 76781.0    MEAN TOTAL CPM =  77080.5
PREDICTED M/L = 5.000E-09   EXPERIMENTAL M/L= 4.768E-09   +/-  5.240E-11

 1510.0
 1489.0
 1402.0  MEAN CPM =  1467.0  MEAN BOUND M/L= 1.613E-10  +/- 1.259E-11

 1246.0
 1197.0
MEAN NSB CPM = 1221.5  MEAN BD.(FM/ML INCUBATION)= 27.0    +/- 7.6
MEAN BD. (FM/MG PROTEIN) = 19.1
0
----------------------------------------

 32380.0
 30400.0    MEAN TOTAL CPM =  31390.0
PREDICTED M/L = 2.500E-09   EXPERIMENTAL M/L= 1.942E-09   +/-  1.732E-10

 934.0
 895.0   MEAN CPM =  914.5   MEAN BOUND M/L= 1.006E-10  +/- 6.065E-12

 704.0
 691.0
 693.0
MEAN NSB CPM = 696.0   MEAN BD.(FM/ML INCUBATION)= 24.6    +/- 1.5
MEAN BD. (FM/MG PROTEIN) = 17.0
0
----------------------------------------
 16681.0
 16565.0    MEAN TOTAL CPM =  16623.0
PREDICTED M/L = 1.250E-09   EXPERIMENTAL M/L= 1.028E-09   +/-  1.015E-11

 396.0
 433.0   MEAN CPM =  414.5   MEAN BOUND M/L= 4.558E-11  +/- 5.754E-12

 264.0
 279.0
MEAN NSB CPM = 271.5   MEAN BD.(FM/ML INCUBATION)= 15.7    +/- 2.3
MEAN BD. (FM/MG PROTEIN) = 11.1
0
----------------------------------------

        N              FM BOUND/ML INC.              B/F

     1.000000            24.303000                0.005123
     2.000000            29.692000                0.006267

MEAN X = 26.997500  MEAN Y = 0.005695

     3.000000            23.147900                0.012066
     4.000000            24.577500                0.012821
     5.000000            24.357600                0.012704

MEAN X = 24.027600  MEAN Y = 0.012530

     6.000000            16.550600                0.016360
     7.000000            14.901000                0.014705
```

MEAN X = 15.725800 MEAN Y = 0.015533

N	FM BOUND/ML INC.	B/F
1.000000	26.997500	0.005695
2.000000	24.027600	0.012530
3.000000	15.725800	0.015533

THE EQUATION FOR A STRAIGHT LINE IS: Y = A + BX

B = SLOPE OF REGRESSION LINE =-7.62303E-04

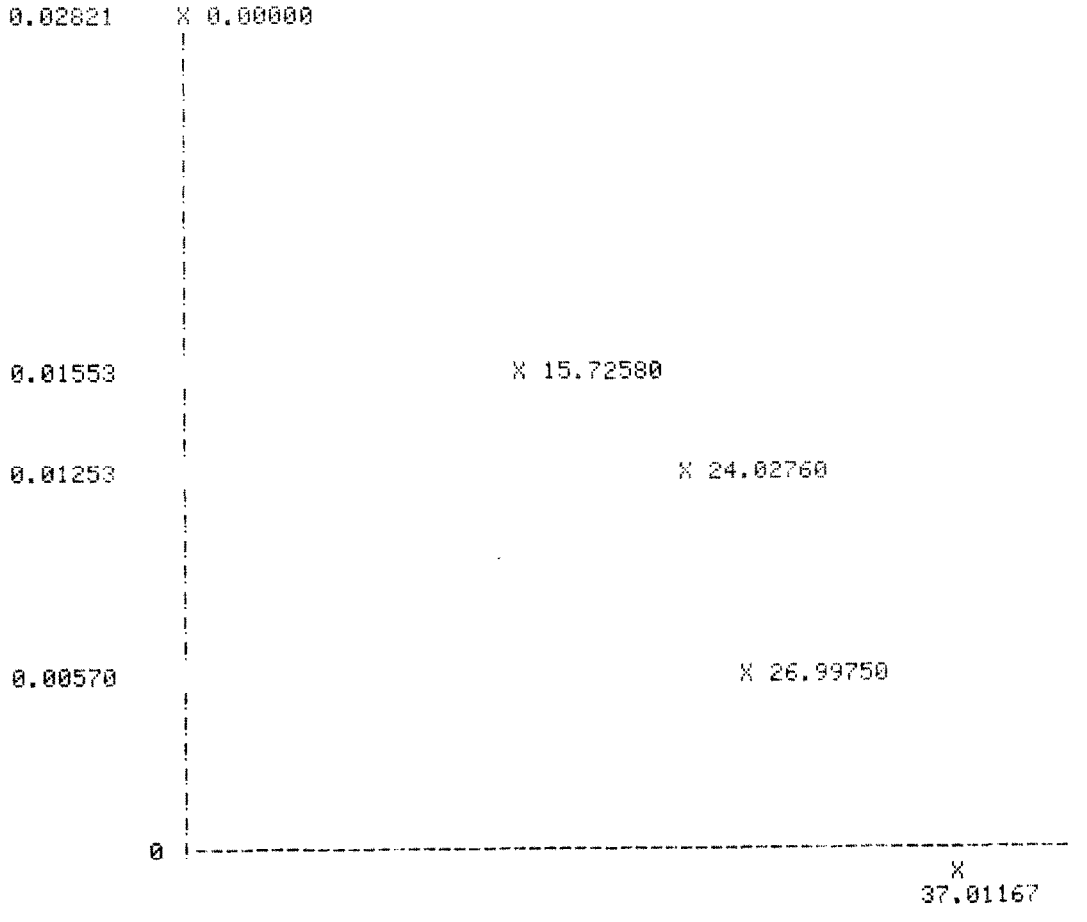
K(D) = 1.31191E-09 MOLES/L

A = THE Y INTERCEPT OF THE LINE = 0.02821

-A/B = THE X INTERCEPT OF THE LINE = 37.01167 FM/ML

THE NUMBER OF BINDING SITES, N = 37.01167 FM/ML INCUBATION VOLUME OR 26.19368 FM/MG PROTEIN OR 15776453871.80000 SITES/MG

R = THE REGRESSION COEFFICIENT = -0.98334



DISCUSSION

The quantitation of binding capacity of specific tissue protein receptors is facilitated by rapid data processing systems. A Hewlett–Packard model 9830A calculator with 9866A printer was utilized to develop a simple but highly flexible program capable of dealing with a variety of clinical and research problems associated with tissue receptor assays. The program is designed to permit considerable operator control over data input without demanding extensive technical knowledge of either computer systems or the mathematics of receptor binding assays. The format comprises a compact presentation of raw data and analytical results which is highly convenient in maintaining records of both clinical and research results.

In a very brief period raw binding data can be converted into a finished Scatchard analysis with all parameters tabulated. Very significant advantages of such a system aside from remarkable efficiency include the following: First, mathematical errors in what are largely tedious calculations are eliminated. Second, through extensive editing capabilities the data can be examined after the deletion or modification of any element. Third, the conversational format of the program allows its use by individuals not fully appreciative of theoretical aspects of binding data. Fourth, a written output of computations and results in standard format facilitates quality control and record keeping.

Adaptation of the program to a number of other calculator systems which have a somewhat greater or lesser memory capacity, different input or output accessories such as card or tape readers or plotters, or which employ a different language is readily feasible. The particular system and format discussed here has proven to be extrememly efficient in terms of saving labor and relieving the tediousness of data reduction.

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