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BASIC COMPUTER PROGRAM FOR AMINO ACID ANALYSIS DATA*

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SUMMARY

A computer program for the reduction of output data from automatic amino acid analyzers has been written using the Beginners all Purpose Symbolic Instruction Code (BASIC) language. From input data consisting of integrator peak areas and sample weight and volume the program calculates μmoles of amino acids present in the run, total μmoles amino acids in the sample, minimal molecular weight, residue number composition, μmoles amino acid per milligram sample, grams amino acid per 100 g sample (% yield), and grams amino acid per 100 g protein.

METHODS

A Beckman Model 120 C automatic amino acid analyzer equipped with an infotronics CRS-110A digital integrator was used for all amino acid analyses. Two column methodology of Spackman *et al.*[1] was employed and both columns were equipped with Beckman manual sample injectors and adjustable column fittings. Both the long and short column sample injection loops were calibrated to contain 1.05 ml. Standard peak areas (color values) were obtained by analyzing a 0.1 μmole/ml amino acid solution prepared from the Beckman standard.

Protein hydrolyses were performed in 5.7 *N* HCl in sealed evacuated tubes at 105° for 24 h. After hydrolysis the samples were evaporated to dryness in a vacuum desiccator and dissolved in citrate sample buffer at pH 2.2. The samples were filtered through a millipore filter prior to analysis. No corrections were made for water or ash content, or for incomplete or destructive hydrolysis.

A Xerox Data System Sigma 7 computer operating in the Universal Time Sharing (UTS) system was utilized for building and executing the program. All operations were performed at a satelite teletype terminal via an acoustic data coupler. When not in use, the program was stored on punched paper tape and/or magnetic disc in the central computer.

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PROGRAM DESCRIPTION

The appearance of amino acid analyzers capable of multiple successive analyses and on-line electronic digital integrators has made it possible to perform large numbers of analyses in short periods of time. The limiting factor in such rapid procedures has become the calculation and reduction of output data in many instances. This computer program greatly reduces the time involved in the processing of analytical data, and in addition the Beginners all Purpose Symbolic Instruction Code (BASIC) language promotes facile comprehension by individuals not highly trained in computer technology.

Several other computer programs have been written for the treatment of amino acid analysis data[2–6]. Most, however, are complex, limited in their application and the information they yield, or they are written for specially designed machines. The present program is written in the elementary BASIC language[7], which is most effective in small to medium scale applications of a computational nature. Program modifications and adjustments are easily performed should circumstances require it. The program is compatible with any computer that accepts the BASIC language, such as the Sigma 7 used in this study.

The program is loaded into the computer from punched paper tape (15 min required) or from magnetic disc in the central facility upon command from the terminal operator (seconds required). After loading the operator types in the integrator peak areas for individual amino acids and the analysis run number and then initiates execution. No punch cards or data conversions are needed. Four subroutines are incorporated into the program and are called up by the operator as required. The program pauses after execution of a subroutine until another command is input. Individual subroutines are discussed below. The complete program is given in the Appendix.

*Subroutine 1: calculation of μmoles amino acid present in the run, command [0,0,0]**

To execute subroutine 1 the operator types [0,0,0]. The computer calculates the μmoles of amino acid present from the ratio of the experimental to standard peak areas in steps 750–860 according to:

$$\mu\text{mole amino acid} = \frac{\text{area experimental peak}}{\text{area standard peak}} \cdot 0.105\ \mu\text{mole}$$

The values are output by the computer in steps 120–225. Step 245 then halts execution until another command is input by the operator.

Subroutine 2: calculation of residue numbers, command [0,0,1]

Upon receipt of this command the computer requests the operator to input a selected division factor. The computer then divides the μmole amount of each amino acid (as calculated in subroutine 1) by the requested division factor in steps 1125–1160 and prints out the results in steps 1175–1340. An indication of amino acid composition relative to the content of the amino acid chosen as the division factor is obtained.

* Operator input is designated with brackets. Computer output is indicated with capitals.

Normally, the rarest amino acid is arbitrarily assigned a residue value of 1. Subroutine 2 also sums and prints the total number of amino acids present, excluding ammonia, and calculates an approximate molecular weight for a protein composed of this number of amino acid residues through multiplication by 110, an arbitrarily selected average residue molecular weight. Examination of the relative amounts of amino acids and/or comparison of the calculated molecular weight with that obtained from a different method indicates the correctness of the original assignment. The operator may then wish to repeat subroutine 2 selecting a different division factor.

Subroutine 3: refined calculation of residue numbers and amino acid composition, command [0,1,0]

This subroutine (steps 250–410) is essentially a refinement of subroutine 2 and calculates "best fit" residue numbers based on averaged division factors of up to ten amino acids that are assigned integral values by the operator (from the output of subroutine 2). This allows relative residue numbers to be based on yields of several amino acids rather than on a single choice as in subroutine 2. Integral residue number assignments for selected amino acids must be input by the operator in steps 395, 400 and 405 before execution of the subroutine. The μmole yield of up to five amino acids (from subroutine 1) is entered in each of steps 395 and 400. If less than five amino acids are entered, zeros must be input to give a total of 5 numbers in each step. In step 405 the assigned integral residue numbers (estimated from subroutine 2) corresponding to the chosen amino acids in steps 395 and 400 are input. For each zero in steps 395 and 400 the number 1 is entered in step 405. The total number of different amino acids used in steps 395 and 400 is then entered last. Step 405 should therefore have a total of eleven numbers.

Upon execution, subroutine 3 prints out μmoles amino acid (repeat of subroutine 1 output) and residue number based on an averaged division factor calculated from the yields and assigned integral numbers of amino acids input in steps 395–405. The numerical value of that averaged factor plus the total number of amino acids plus the approximate molecular weight is also printed.

Subroutine 4: quantitative expression of analytical data, command [1,0,0]

Several calculative subroutines are actually incorporated into this portion of the program. They are performed sequentially and result in calculation of total μmoles amino acid in the original hydrolysate, μmoles amino acid per milligram sample, percentage composition (grams amino acid per 100 g sample), total recovery in grams amino acid per 100 g sample, and percentage composition based on the sample as 100% protein (grams amino acid per 100 g protein). After the command [1,0,0] is given, the operator is requested to input in step 425 the sample volume, *i.e.* the volume of the sample buffer containing the dissolved hydrolysate, and the milligrams of sample hydrolyzed. Subroutine 4 is contained in steps 420–745 and 865–1120. The first calculation determines the total μmoles of amino acids in the sample (steps 865–940). The μmole yield previously calculated in subroutine 1 is multiplied by the ratio of the total sample volume to the volume analyzed (1.05 ml), *i.e.*

$$\text{total } \mu\text{mole} = \mu\text{mole yield} \cdot \frac{\text{total sample volume}}{1.05 \text{ ml}}$$

Micromoles per milligram sample is calculated in steps 945–980 by dividing the total μmoles by the mg of sample hydrolyzed, *i.e.*

$$\frac{\mu\text{mole}}{\text{mg sample}} = \frac{\text{total } \mu\text{moles}}{\text{total mg sample}}$$

Grams of amino acid per 100 g sample (percentage composition) is calculated in steps 985–1080 according to

$$\frac{\text{g amino acid}}{\text{100 g sample}} = \frac{\mu\text{mole amino acid}}{\text{mg sample}} \cdot \text{residue molecular weight} \cdot 0.1$$

The total sample recovery is then computed by summing the percentage composition for each amino acid. This total yield value is next utilized in steps 1085–1120 to calculate the percentage composition of each amino acid based on the sample as 100% protein

$$\%\text{ composition} \cdot \frac{100}{\text{total recovery}} = \%\text{ composition based on samples as 100\% protein}$$

This last treatment is useful in adjusting for possible yield losses (due to water, ash content, manipulative losses, etc.) when dealing with pure proteins and for determination of protein quality, *i.e.* distribution of protein amino acids in heterogenous samples such as food stuffs and agricultural materials. If the sample weight is unknown, subroutine 4 is not used. Likewise, if heterogenous samples are analyzed, subroutines 2 and 3 are unused since residue composition would have no meaning.

PROGRAM EXECUTION

After the program has been loaded into the computer and before execution is initiated, the operator types in the integrator peak areas in statements 375 and 380. Step 375 receives the peak areas for lysine, histidine, ammonia, arginine, aspartic acid, threonine, serine, glutamic acid and proline. Peak areas for glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine are typed into step 380. Standard peak areas (color yields) from a previous standardization run are similarly typed into steps 385 and 390. The standard peak areas are retained in the program and need not be reentered for subsequent program executions unless the operator wishes to change their value. The run number or other sample identification is typed into step 15. No additional data statements are required for execution of subroutines 1, 2 and 4.

Execution is initiated by typing [RUN]. The computer then requests from the operator a "subroutine selection" and halts until the appropriate command is entered. Upon receiving the command, the selected subroutine is executed, data are output, and the program pauses until another command is received.

RESULTS

Results obtained through use of the program are demonstrated by the following example of the amino acid analysis of bovine insulin. Actual data input and computer

output are reproduced below. Authorial commentary is included for purposes of clarity.

Commentary: The program has been loaded. Operator types integrator data into steps 375, 380, 385, 390 and the run number into step 15.

Input: 15 Z5 = 37372
375 DATA 14.44, 26.55, 47.82, 12.52, 37.03, 12.01, 35.58, 91.24, 3.05
380 DATA 53.56, 37.05, 32.40, 48.15, 0, 5.59, 81.29, 51.84, 37.50
385 DATA 47.74, 45.49, 24.60, 40.71, 40.98, 41.15, 41.34, 39.95, 9.79
390 DATA 41.72, 41.12, 19.21, 38.89, 41.34, 41.79, 43.24, 42.54, 42.11
RUN

Output: 10:15 Nov 29 DALE...
BASIC PROGRAM TO EVALUATE AMINO ACID ANALYSIS
THE AAA RUN NO. IS 37372
INPUT VALUES OF U6, U7, Z4(SUBROUTINE SELECTION)?*

Input: 0,0,0

Output:

	MICROMOLES AMINO ACID	RESIDUE NUMBER
LYS	3.12232E-02	0
HIS	6.25477E-02	0
NH4	0.204110	0
ARG	3.08809E-02	0
ASP	9.01287E-02	0
THR	2.96648E-02	0
SER	8.62994E-02	0
GLU	0.221661	0
PRO	3.33594E-02	0
GLY	0.129491	0
ALA	9.04079E-02	0
CYS	0.167586	0
VAL	0.127509	0
MET	0	0
ILE	1.29085E-02	0
LEU	0.179542	0
TYR	0.122650	0
PHE	9.38394E-02	0

245 HALT

Commentary: From this output it appears that lysine, arginine, threonine, and proline are the least abundant residues and are approximately equal in amount. Arbitrarily arginine is assigned a residue number of 1 and is used as the division factor in the next subroutine**.

* The preceding output is generated each time [RUN] is input. Only the question mark will be indicated hereafter.

** The lesser yield of isoleucine is ignored in light of the 24-h hydrolysis time.

Input: RUN
Output: ?
Input: 0,0,1
Output: INPUT VALUE OF SELECTED DIVISION FACTOR?
Input: 0.03088

Output: RESULTS OF DIVISION FACTOR

LYS	1.01111
HIS	2.02551
NH4	6.60977
ARG	1.00003
ASP	2.91867
THR	0.960647
SER	2.79467
GLU	7.17815
PRO	1.08029
GLY	4.19337
ALA	2.92772
CYS	5.42701
VAL	4.12919
MET	0
ILE	0.418022
LEU	5.81420
TYR	3.97182
PHE	3.03884

THE SUM OF AMINO ACIDS EXCLUDING NH4 IS 48.8893
THE APPROXIMATE MOLECULAR WEIGHT IS 5377.82
410 HALT

Commentary: The output above from subroutine 2 has generated the residue ratio of all other amino acids to arginine which was assigned a value of 1. Observation indicates that several residues approach integral values. Several of these residues and their assumed integral values are used for the refinement procedure in subroutine 3.

Input: 395 DATA 0.03122, 0.06255, 0.03088, 0.09013, 0.02866
400 DATA 0.03336, 0.09041, 0.12265, 0.09384, 0
405 DATA 1,2,1,3,1,1,3,4,3,1,9
RUN

Commentary: The residues chosen for computation of the averaged division factor, and their assigned integral values are; lysine, 1; histidine, 2; arginine, 1; aspartic acid, 3; threonine, 1; proline, 1; alanine, 3; tyrosine, 4; phenylalanine, 3. Total number of different residues averaged is 9.

Output: ?
Input: 0,1,0

Output:

AMINO ACID	MICROMOLES	RESIDUE NUMBER	(LITERATURE)
LYS	3.12232E-02	1.01258	1
HIS	6.25477E-02	2.02845	2
NH4	0.204110	6.61936	6
ARG	3.08809E-02	1.00148	1
ASP	9.01287E-02	2.92291	3
THR	2.96648E-02	0.962040	1
SER	8.62994E-02	2.79872	3
GLU	0.221661	7.18856	7
PRO	3.33594E-02	1.08186	1
GLY	0.129491	4.19945	4
ALA	9.04079E-02	2.93196	3
CYS	0.167586	5.43489	6
VAL	0.127509	4.13518	5
MET	0	0	0
ILE	1.29085E-02	0.418628	1
LEU	0.179542	5.82263	6
TYR	0.122650	3.97758	4
PHE	9.38394E-02	3.04325	3
			51

TOTAL NUMBER AMINO ACIDS EXCLUDING NH4 48.9602
AVERAGE DIVISION FACTOR VALUE IS 3.08353E-02
APPROXIMATE MOLECULAR WEIGHT OF THE PROTEIN 5385.62
410 HALT

Commentary: The program has now generated residue numbers based on yields of several selected amino acids as opposed to one selected value in subroutine 2. The averaged division factor and the approximate molecular weight are also output. The known composition of bovine insulin is included for comparison[8].

Input: RUN
Output: ?
Input: 1,0,0
Output: INPUT VALUES OF B8 and B9-VOL SAMPLE, MG SAMPLE?
Input: 10.0, 2.1
Commentary: The sample volume and weight of insulin hydrolyzed were 10.0 ml and 2.1 mg, respectively.
Output: AMINO ACID ANALYSIS OF PROTEIN SAMPLE

	TOTAL MICROMOLES	MICROMOLES PER MILLIGRAM SAMPLE	% COMPOSITION SAMPLE
LYS	0.297364	0.141602	1.81534
HIS	0.595692	0.283663	3.72166
NH4	1.94390	0.925668	1.57364

ARG	0.294104	0.140049	2.18757
ASP	0.858368	0.408747	4.70467
THR	0.282522	0.134534	1.36014
SER	0.821189	0.391380	3.40892
GLU	2.11106	1.00527	12.9780
PRO	0.317708	0.151290	1.46902
GLY	1.23325	0.587261	3.35326
ALA	0.861027	0.410013	2.91519
CYS	1.59606	0.760028	15.8086
VAL	1.21438	0.578274	5.73070
MET	0	0	0
ILE	0.122938	5.85420E-02	0.662695
LEU	1.70993	0.814252	9.21733
TYR	1.16809	0.556235	9.07776
PHE	0.893708	0.425575	6.26447

TOTAL RECOVERY IN GRAMS PER 100 GRAM SAMPLE 86.2489

% COMPOSITION BASED ON SAMPLE AS 100% PROTEIN

LYS	2.10476
HIS	4.31502
NH4	1.82453
ARG	2.53635
ASP	5.45476
THR	1.57699
SER	3.95242
GLU	15.0471
PRO	1.70324
GLY	3.88789
ALA	3.37997
CYS	18.3290
VAL	6.64437
MET	0
ILE	0.768352
LEU	10.6869
TYR	10.5251
PHE	7.26324

AMOUNT OF RECOVERY BASED ON 100% PROTEIN 100.000
745 HALT
SYSTEM
BYE

Commentary: In this subroutine the program calculates quantitative aspects of the analysis as indicated. SYSTEM and BYE represent termination of execution.

DISCUSSION

Throughout the computer program attempts have been made to allow effective treatment of several different types of calculative procedures typically encountered in amino acid analyses. A choice of subroutines allows the operator to select the type of calculation and output data presentation which best suits the analytical problem. Subroutines 1 and 4 represent the quantitative aspects of the program for calculations based on the weight of sample hydrolyzed. Subroutines 2 and 3 are by comparison qualitative in that the program generates protein composition data based on arbitrary choices by the operator. In cases where sample weight and/or molecular weight are unknown, the operator, through examination of generated residue numbers, may approximate a minimal amino acid composition and therefore a minimal value for molecular weight. Comparison with molecular weight data from different methods allows estimation of the total amino acid composition. These approximating procedures can be very helpful in the early stages of protein isolation and characterization. In cases of chemical modification studies the absence or reduction of affected residues can quickly be detected through generation of residue numbers based on yields of several non-susceptible amino acids.

The determination of total recovery in addition to the standard weight composition data in subroutine 4 indicates the extent to which the sample is contaminated with non-protein material. Adjustment for possible low yields or non-protein contaminants are made in subroutine 4 when % composition based on the sample as 100% protein is calculated. Expression of the data in this fashion is particularly useful when determining the protein quality, *i.e.* amino acid distribution in foodstuffs and agricultural samples.

The program, as written, does not incorporate correction values for possible losses of serine or threonine, nor is tryptophan calculated due to destruction during acid hydrolysis. Although corrective factors for serine and threonine could be included, our experience has shown that the extent of destruction is sample dependent and therefore not validly characterized by a single correction factor. Extrapolation to zero time hydrolysis from several analyses would likely give the best values for threonine and serine. For similar reasons no attempts were made to correct for slowly hydrolyzing residues such as isoleucine and valine.

The BASIC language contains no provisions for rounding numbers and as a consequence the output data must not be interpreted beyond the significance of the input data.

The program is written to accept up to eighteen amino acids and thus accounts for all normal acids found in protein hydrolysates. Unusual amino acids may be calculated so long as the total number of all amino acids is not greater than eighteen. The appropriate standard peak area (color value) in steps 385 or 390 must be changed so that it corresponds correctly with the unusual amino acid. For example, a cysteic acid peak area may be input in the position of cystine and the appropriate standard peak area inserted for calculation. The new residue weight for the modified amino acid would have to be inserted in the appropriate step, in this case step 1045, if subroutine 4 were executed. The only changes in the program which might be required for use by other workers would be the column sample volume. The amino acid analyzer used here had short and long column sample volumes of 1.05 ml, and this

value is incorporated into the calculations. If a different sample volume is used (or different concentration of the standard amino acid solution), appropriate changes are easily made in steps 800, 850, 885, and 930. No other changes should be necessary for general usage of the program. Extensive modification, if desirable, may be easily performed in view of the BASIC language employed.

ACKNOWLEDGEMENTS

The valuable advice and discussions provided by Dr. David Smith are appreciated. This work was supported by a Research Grant from the Research Corporation and by the Montana Agricultural Experiment Station.

APPENDIX

The BASIC computer program described in this paper is reproduced below. A copy will be provided upon request to the authors.

```
0020:   HAPNER,ROB    11/25/73    14:05
5 PRINT 'BASIC PROGRAM TO EVALUATE AMINO ACID ANALYSIS'
10 REM LOAD AAA RUN # VALUE INTO Z5
15 Z5=39473
20 PRINT TAB(12),'THE AAA RUN # IS';Z5
25 FOR N=1 TO 9
30 READ A(N)
35 NEXT N
40 FOR N=1 TO 9
45 READ B(N)
50 NEXT N
55 GOSUB 750
60 E(1),E(2),E(3),E(4),E(5),E(6),E(7),E(8),E(9),D=0
65 F(1),F(2),F(3),F(4),F(5),F(6),F(7),F(8),F(9)=0
70 PRINT
75 PRINT'INPUT VALUES OF J6,J7,Z4 (SUBROUTINE SELECTION)'
80 INPUT J6,J7,Z4
85 IF J7=1 GOTO 250
90 IF Z4=1 GOTO 1125
95 IF J6=1 GOTO 415
100 PRINT
105 PRINT
110 PRINTUSING 120
115 PRINTUSING 125
120 :       MICROMOLES          RESIDUE
125 :       AMINO ACID          NUMBER
130 PRINT '*****************************************'
135 PRINT
140 PRINT 'LYS';C(1),TAB(23);E(1)
145 PRINT 'HIS';C(2),TAB(23);E(2)
150 PRINT 'NH4';C(3),TAB(23);E(3)
155 PRINT 'ARG';C(4),TAB(23);E(4)
160 PRINT 'ASP';C(5),TAB(23);E(5)
165 PRINT 'THR';C(6),TAB(23);E(6)
170 PRINT 'SER';C(7),TAB(23);E(7)
175 PRINT 'GLU';C(8),TAB(23);E(8)
180 PRINT 'PRO';C(9),TAB(23);E(9)
185 PRINT 'GLY';D(1),TAB(23);F(1)
190 PRINT 'ALA';D(2),TAB(23);F(2)
195 PRINT 'CYS';D(3),TAB(23);F(3)
200 PRINT 'VAL';D(4),TAB(23);F(4)
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```
205 PRINT 'MET';D(5),TAB(23);E(5)
210 PRINT 'ILE';D(6),TAB(23);E(6)
215 PRINT 'LEU';D(7),TAB(23);E(7)
220 PRINT 'TYR';D(8),TAB(23);E(8)
225 PRINT 'PHE';D(9),TAB(23);E(9)
230 IF Z4=1 GOTO 410
235 IF U7=P GOTO 340
240 PRINT
245 PAUSE
250 READ U1,U2,U3,U4,U5,P1,P2,P3,P4,P5
255 READ S1,S2,S3,S4,S5,S6,S7,S8,S9,S,T
260 R=(U1/S1+U2/S2+U3/S3+U4/S4+U5/S5)
265 C=(P+(P1/S6+P2/S7+P3/S8+P4/S9+P5/S))/T
270 N,S=0
275 FOR S=1 TO 9
280 N=N+1
285 E(S)=C(N)/C
290 F(S)=D(N)/C
295 NEXT S
300 N,K1,K2=0
305 FOR N=1 TO 9
310 K1=K1+E(N)
315 K2=K2+F(N)
320 NEXT N
325 K3=K2+K1: K4=120*(K3-E(3))
330 U7=U7+1
335 GOTO 100
340 PRINT
345 PRINT 'TOTAL NUMBER AMINO ACIDS EXCLUDING NH4';K3-E(3)
350 PRINT
355 PRINT
360 PRINT 'AVERAGE DIVISION FACTOR VALUE IS';C
365 PRINT
370 PRINT' APPROXIMATE MOLECULAR WEIGHT OF THE PROTEIN';K4
375DATA77.92,84.96,82.35,87.80,129.41,142.25,98.97,41.74,23.97
380DATA 37.51,73.44,135.43,26.43,0,73.48,39.23,40.42,36.32
385DATA48.56,44.57,24.60,42.57,43.14,42.51,43.29,43.22,9.60
390DATA43.43,43.03,20.30,39.65,46.21,45.47,47.54,44.38,41.96
395 DATA .08151,.07962,.24981,.16727,.11196
400 DATA .03936,0,0,0,0
405 DATA 2,2,6,4,3,1,1,1,1,6
410 END
415 PRINT
420 PRINT'INPUT VALUES OF B8 AND B9=VOL SAMPLE, MGS SAMPLE'
425 INPUT B8,B9
430 GOSUB 865
435 GOSUB 945
440 GOSUB 985
445 N,K5=0
450 FOR N=1 TO 9
455 K5=K5+J(N)+V(N)
460 NEXT N
465 PRINT
470 PRINT TAB(14);'AMINO ACID ANALYSIS OF PROTEIN SAMPLE'
475 PRINT '          ************************************'
480 PRINT
485 PRINTUSING 490
490 PRINTUSING 500
495 :   TOTAL                MICROMOLES PER          %COMPOSITION
500 :  MICROMOLES              MICRO SAMPLE              SAMPLE
505 PRINT'============================================================'
510 PRINT 'LYS';G(1);TAB(25);A(1);TAB(50);U(1)
515 PRINT 'HIS';G(2);TAB(25);A(2);TAB(50);U(2)
520 PRINT 'NH4';G(3);TAB(25);A(3);TAB(50);U(3)
525 PRINT 'ARG';G(4);TAB(25);A(4);TAB(50);U(4)
530 PRINT 'ASP';G(5);TAB(25);A(5);TAB(50);U(5)
535 PRINT 'THR';G(6);TAB(25);A(6);TAB(50);U(6)
540 PRINT 'SER';G(7);TAB(25);A(7);TAB(50);U(7)
545 PRINT 'GLU';G(8);TAB(25);A(8);TAB(50);U(8)
550 PRINT 'PRO';G(9);TAB(25);A(9);TAB(50);U(9)
555 PRINT 'GLY';H(1);TAB(25);V(1);TAB(50);V(1)
560 PRINT 'ALA';H(2);TAB(25);V(2);TAB(50);V(2)
565 PRINT 'CYS';H(3);TAB(25);V(3);TAB(50);V(3)
570 PRINT 'VAL';H(4);TAB(25);V(4);TAB(50);V(4)
575 PRINT 'MET';H(5);TAB(25);V(5);TAB(50);V(5)
580 PRINT 'ILE';H(6);TAB(25);V(6);TAB(50);V(6)
585 PRINT 'LEU';H(7);TAB(25);V(7);TAB(50);V(7)
590 PRINT 'TYR';H(8);TAB(25);V(8);TAB(50);V(8)
595 PRINT 'PHE';H(9);TAB(25);V(9);TAB(50);V(9)
600 GOSUB 1085
605 PRINT
610 PRINT 'TOTAL RECOVERY IN GRAMS PER 100 GRAM SAMPLE';K5
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(Continued on p. 110)

(continued)

```
615 PRINT
620 PRINT
625 PRINTUSING 635
630 PRINTUSING 640
635 !      % COMPOSITION BASED ON
640 !      SAMPLE AS 100% PROTEIN
645 PRINT '*****************************'
650 PRINT 'LYS';R(1)
655 PRINT 'HIS';R(2)
660 PRINT 'NH4';R(3)
665 PRINT 'ARG';R(4)
670 PRINT 'ASP';R(5)
675 PRINT 'THR';R(6)
676 PRINT 'SER';R(7)
680 PRINT 'GLU';R(8)
685 PRINT 'PRO';R(9)
690 PRINT 'GLY';T(1)
695 PRINT 'ALA';T(2)
700 PRINT 'CYS';T(3)
705 PRINT 'VAL';T(4)
710 PRINT 'MET';T(5)
715 PRINT 'ILE';T(6)
720 PRINT 'LEU';T(7)
725 PRINT 'TYR';T(8)
730 PRINT 'PHE';T(9)
735 PRINT
740 PRINT 'AMOUNT OF RECOVERY BASED ON 100% PROTEIN';Z8
745 END
750 REM CALCULATION OF MICROMOLES OF AMINO ACID
755 FOR B=1 TO 9
760 READ X(B)
765 NEXT B
770 FOR B=1 TO 9
775 READ Z(B)
780 NEXT B
785 N=B=0
790 FOR B=1 TO 9
795 N=N+1
800 C(B)= (A(N)/X(N))*.105
805 NEXT B
835 N=B=0
840 FOR B=1 TO 9
845 N=N+1
850 D(B)=(B(N)/Z(N))*.105
855 NEXT B
860 RETURN
865 REM CALCULATION OF TOTAL MICROMOLES IN SAMPLE
870 N=B=0
875 FOR B=1 TO 9
880 N=N+1
885 G(B)=C(N)*(88/1.05)
890 NEXT B
915 N=B=0
920 FOR B=1 TO 9
925 N=N+1
930 H(B)=D(N)*(88/1.05)
935 NEXT B
940 RETURN
945 REM CALCULATION OF MICROMOLES PER MILIGRAM SAMPLE
950 N=B=0
955 FOR B=1 TO 9
960 N=N+1
965 W(B)=G(N)/89
970 Y(B)=H(N)/89
975 NEXT B
980 RETURN
985 REM CALCULATION OF GRAMS AMINO ACID PER 100 GRAMS SAMPLE
990 U(1)=W(1)*(128.2)*.1
995 U(2)=W(2)*(131.2)*.1
1000 U(3)=W(3)*(17)*.1
1005 U(4)=W(4)*(156.2)*.1
1010 U(5)=W(5)*(115.1)*.1
1015 U(6)=W(6)*(101.1)*.1
1020 U(7)=W(7)*(87.1)*.1
1025 U(8)=W(8)*(129.1)*.1
1030 U(9)=W(9)*(97.1)*.1
1035 V(1)=Y(1)*(57.1)*.1
1040 V(2)=Y(2)*(71.1)*.1
1045 V(3)=Y(3)*(208)*.1
1050 V(4)=Y(4)*(99.1)*.1
1055 V(5)=Y(5)*(131.2)*.1
1060 V(6)=Y(6)*(113.2)*.1
```

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1065 V(7)=V(7)*(113.2)**1
1070 V(8)=V(8)*(163.2)**1
1075 V(9)=V(9)*(147.2)**.1
1080 RETURN
1085 REM CALC % COMPOSITION BASED ON SAMPLE AS 100% PROTEIN
1090 N,D,Z8=0
1095 FOR 9=1 TO 9
1100 R(9)=U(9)*(100.00/K5)
1105 T(9)=V(9)*(100.00/K5)
1110 Z8=Z8+R(9)+T(9)
1115 NEXT 9
1120 RETURN
1125 REM SUBPR TO CALC COMP BASED ON SELECTED DIV. FACTOR
1130 PRINT
1135 PRINT 'INPUT VALUE OF SELECTED DIVISION FACTOR'
1140 INPUT Z9
1145 FOR N=1 TO 9
1150 M(N)=C(N)/Z9
1155 N(N)=D(N)/Z9
1160 NEXT N
1165 PRINT
1170 PRINTUSING 1175
1175 :  RESULTS OF DIVISION FACTOR
1180 PRINT '--------------------------'
1185 PRINT 'LYS';M(1)
1190 PRINT 'HIS';M(2)
1195 PRINT 'NH4';M(3)
1200 PRINT 'ARG';M(4)
1205 PRINT 'ASP';M(5)
1210 PRINT 'THR';M(6)
1215 PRINT 'SER';M(7)
1220 PRINT 'GLU';M(8)
1225 PRINT 'PRO';M(9)
1230 PRINT 'GLY';N(1)
1235 PRINT 'ALA';N(2)
1240 PRINT 'CYS';N(3)
1245 PRINT 'VAL';N(4)
1250 PRINT 'MET';N(5)
1255 PRINT 'ILU';N(6)
1260 PRINT 'LEU';N(7)
1265 PRINT 'TYR';N(8)
1270 PRINT 'PHE';N(9)
1275 N,9,Z6=0
1280 FOR 8=1 TO 9
1285 N=N+1
1290 Z6=Z6+M(N)+N(N)
1295 NEXT 9
1300 Z7=Z6-M(3)
1305 PRINT
1310 PRINT 'THE SUM OF AMINO ACIDS EXCLUDING NH4 IS';Z7
1315 PRINT
1320 Z8=Z7*120
1325 PRINT
1330 PRINT 'THE APPROXIMATE MOLECULAR WEIGHT IS';Z8
1335 PRINT
1340 GO TO 230
```

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