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INTERFACING A PROGRAMMABLE ELECTRONIC CALCULATOR WITH AN AUTOMATIC AMINO ACID ANALYZER

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SUMMARY

The rapid calculation of data from an automatic amino acid analyzer by a programmable electronic calculator is now possible. The programmable electronic calculator offers a less expensive and more flexible alternative to large computers. Typically, data in the form of retention times and peak areas are read from a punched tape and converted into final values with a minimum of data handling. A digital integrator, teletypewriter with tape punch and reader, tape editor and printer also are required.

A mathematical program is described that has been devised to convert raw data from a 4-h protein hydrolyzate run into final values. The final printout presents in tabular form each amino acid as a percentage of the sample dry weight and crude protein. The total weight percentage of amino acids and percentage recovery of protein based on Kjeldahl nitrogen are also calculated. Other features of the program include the ability to eliminate up to 20 extraneous peaks from the sample and to correct for sampling errors with the aid of internal standards. Amino acid molecular weights and color constants, which are also calculated, are stored without destruction so as to permit the calculation of an unlimited number of samples from the same set of standards.

INTRODUCTION

The advent of automatic amino acid analysis by separation of the acids on ion-exchange columns followed by colorimetric determination of the individual acids[1–4] has necessitated the development of automatic data handling and calculation. One approach to this has been to digitize absorbance and retention time data on punch tape. A large computer is then used to identify the acids and to calculate areas, concentrations and/or related quantities[5–7]. The other approach makes use of a digital integrator to compute areas and retention times and to direct teletypewriter generation of the appropriate tapes for identification and further calculations by a large computer[8]. For this computer we have found it possible to substitute a programmable electronic calculator (PEC) possessing 2K bytes of core memory. Several advantages

of the PEC include relatively low cost, ease of programming and "debugging", and a high degree of accessibility and flexibility.

This paper describes the equipment and software required for PEC conversion of the output from an automatic amino acid analyzer into final tabulated form. In addition to the instructions specific to the PEC used, a flow diagram of program logic to facilitate conversion from one language into another is also included.

MATERIALS AND METHODS

Amino acids were analyzed on an automatic amino acid analyzer (Model 121, Beckman-Spinco Division, Palo Alto, Calif., U.S.A.)* equipped with an integrator (Model CRS-110A, Infotronics Corp., Houston, Texas, U.S.A.) and a teletypewriter (Model 33ASR, Teletype Corp., Skokie, Ill., U.S.A.). The integrator and teletypewriter were factory modified so that four horizontal rows of holes, or rubouts, are generated on the tape whenever the integrator is reset to zero time. This modification is useful for programming purposes, and also facilitates the separation of tapes by run. Calculations were performed with a programmable electronic calculator (Model 720 B, Wang Laboratories, Tewksbury, Mass., U.S.A.) equipped with a tape editor (Model 703) and an output writer (Model 701 A). Instructions to be inserted in the PEC are given in the Appendix.

The calibration mixture used (Type I standard calibration mixture) was obtained from Beckman Instruments. Two additional amino acids, α-amino-β-guanidinopropionic acid hydrochloride (β-AGP) and L-norleucine (Nle) (Pierce Chemical Co., Rockford, Ill., U.S.A.) were added to the calibration mixture to serve as internal standards. Also, L-cysteic acid monohydrate (Pierce Chemical Co.) was added to the calibration mixture. All standard amino acids were run at an initial concentration of 0.5 μmole/ml. The standard 4-h protein hydrolyzate methodology described by Beckman Instruments was used for all analyses[9].

Ten different varieties of triticale, a hybrid grain of wheat and rye, were analyzed during development of this program. The grain was obtained through the courtesy of Dr. Darrell Morey, University of Georgia College of Agriculture, Coastal Plains Station, Tifton, Ga., U.S.A. The triticale was ground to pass a 40-mesh screen and ball-milled for 24 h. A 100-mg sample was weighed into an ampoule and 10 ml of 6 *N* HCl added. The sample was degassed and sealed under vacuum. After hydrolysis for 22.5 h at 110°, the contents of the ampoule were filtered by suction through a medium-pore sintered-glass filter, and twice evaporated to dryness. After the first evaporation, the residue was dissolved in deionized water, and after the second, in citrate buffer of pH 2.2. Next, the sample was chilled and filtered through a 0.45-μm MF Millipore filter (Millipore Corp., Bedford, Mass., U.S.A.). β-AGP and Nle were present in the citrate buffer at a concentration of 0.5 μmole/ml.

EXPLANATION OF PROGRAM

The program was designed with four functions in mind: (1) to accept input data from both punched tape and keyboard; (2) to identify only those peaks common to

* Mention of commercial items does not imply endorsement by the U.S. Department of Agriculture over others of a similar nature.

both sample and calibration standard; (3) to perform calculations; and (4) to print out answers in tabular form.

Virtually all of the necessary data are generated automatically on punched tape. Exceptions are four values which may change for each run and must be entered manually through the calculator keyboard. In addition, it is necessary to have a punched tape containing the molecular weights of the standard amino acids in

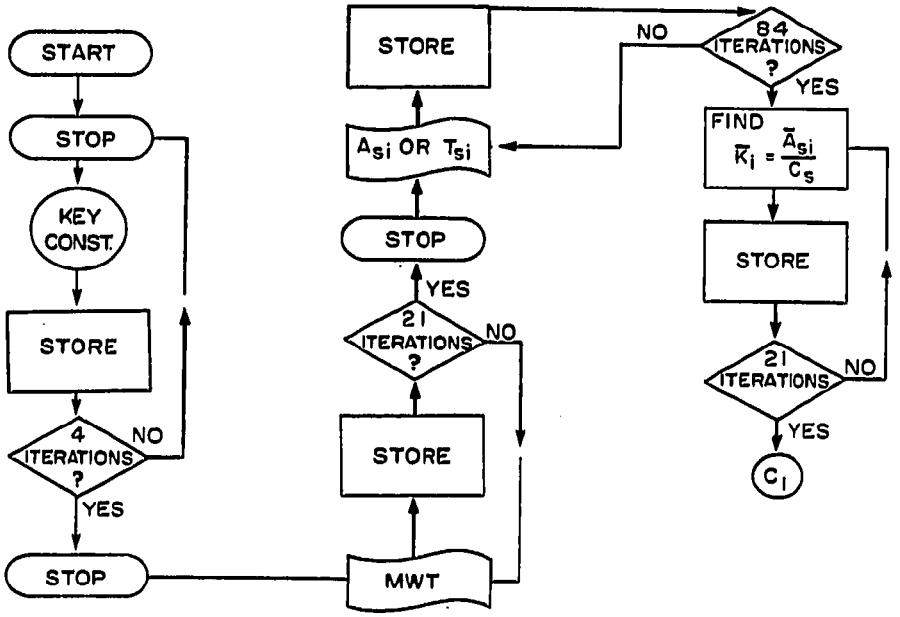

Fig. 1. Flow diagram of data entry and calculation of color constants.

ascending order of elution time. The first column in Fig. 1 shows the logic for keying the range, sample dry weight, percentage Kjeldahl nitrogen and dilution volume of the dried hydrolyzed residue. The range (R), which is the deviation between standard and sample peaks, expressed in minutes, enables the peaks of the sample to be compared with peaks in the calibration standard:

$$|\bar{T}_{si} - T_{uj}| \leqslant R \tag{1}$$

where $\bar{T}_{si}$ is the average retention time in minutes of the ith standard peak and T_{uj} that of the jth unknown peak. If this condition holds, the unknown is identified as the standard. A similar method has been used previously in other computer programs[8] except that an R value has been assigned to each peak and extraneous or unknown peaks are calculated with a standard color constant. It has been found by experiment that one R value (3 min) permits the correct concentration to be determined for better than 95% of the peaks. If an unidentifiable peak (T_{xk})* satisfies the conditions 2 and 3

* For the purposes of this paper, an "unidentifiable" peak is one which is not included in the calibration mixture.

$$T_{xk} < T_{uj} \tag{2}$$

$$|T_{xk} - \bar{T}_{si}| \leqslant R \tag{3}$$

its area (A_{xk}) will be used in calculations rather than the area of the unknown peak (A_{uj}). Nonetheless, the flexibility of the PEC allows this problem to be solved easily. To eliminate the unidentifiable peak, one indexes a different time, replacing T_{xk} in storage with a value that does not satisfy condition 3.

The second and third columns in Fig. 1 depict the entry and storage of molecular weights (MWT), areas (A_{si}) and retention times of standard amino acids and the calculation of an average color constant, $\bar{K}_i$, based on two standards according to eqns. 4 and 5:

$$\bar{A}_{si} = A_{si(1)} + A_{si(2)} \tag{4}$$

$$\bar{K}_i = \bar{A}_{si}/C_{si} \tag{5}$$

Eqn. 4 applies only if the concentration of the standard (C_{si}) is 0.5 units (μmole/ml in this work). Under these conditions, additions of areas directly into storage yield the area of unit concentration. We have found that analysis of one standard, four samples and another standard in that order yields results which are unaffected by ninhydrin deterioration. The circled, subscripted C in Figs. 1–4 indicates where the end of the flow diagram in one figure joins the beginning of another in the next figure.

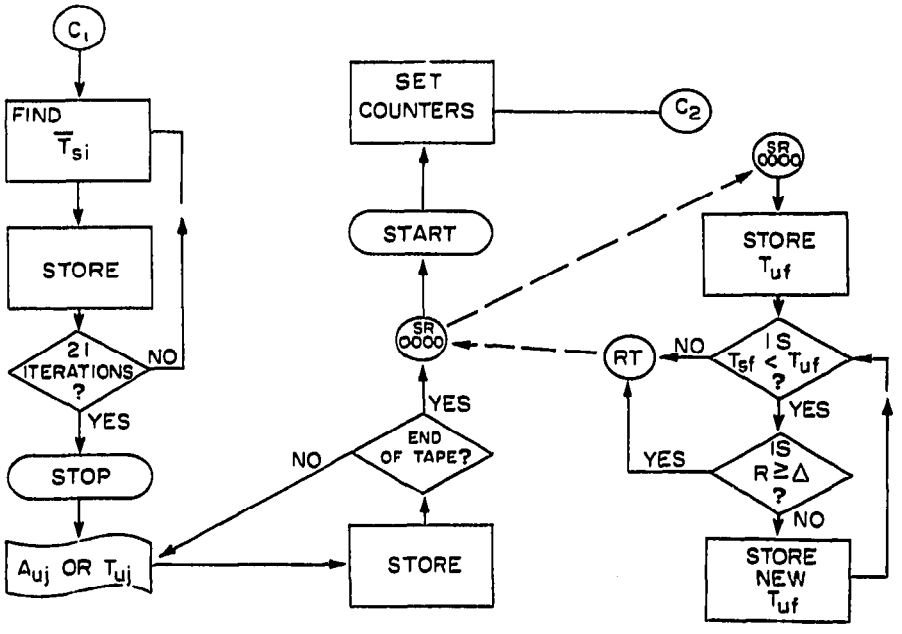

Fig. 2. Flow diagram of data entry and editing.

In addition to the tape entry and storage of unknown peak areas (A_{uj}), the first two columns in Fig. 2 depict instructions for obtaining an average retention time for duplicate runs of the calibration standard ($\bar{T}_{si}$). The four rubouts at the end of the

unknown tape signal the PEC to perform Subroutine 0000 (SR 0000). This subroutine, shown in the last column in Fig. 2, eliminates all unidentifiable peaks greater than the final calibration standard peak ($\bar{T}_{sf}$) and places in storage the retention time of the final identifiable unknown peak (T_{uf}).

Fig. 3 illustrates the logic for identifying peaks to be calculated. Singly, in chronological order, each T_{uj} is recalled from storage and checked for identity with T_{uf}. If the times do not match, T_{uj} is examined to see if it satisfies condition 1 (see upper left-hand branch of Fig. 3). In the event that it does not, the unknown retention time is not considered to be identical with the calibration standard peak with which it is being compared. In this case, a check is made to determine whether condition 6 is satisfied:

$$T_{uj} - \bar{T}_{si} + R \geqslant 0 \tag{6}$$

Should condition 6 be satisfied, the retention time of the unknown is higher than that of the calibration standard, and no unknown peak exists in the sample which is identical with the standard. SR 109 and SR 110 are then called upon to increment, by one, the counters controlling the $\bar{T}_{si}$ and $\bar{K}_i$ to be used in the next comparison and calculation. Further, zero is placed in storage for the concentration of the missing amino acid and SR 108 advances the answer counter by one. Should condition 6 not be satisfied, the retention time of the unknown is less than that of the calibration standard, and the peak cannot be identified. Consequently, SR 111 and SR 112 cause unit increment of counters which control the T_{uj} and A_{uj} to be used in the next comparison and calculation. However, if the T_{uj} under consideration satisfies con-

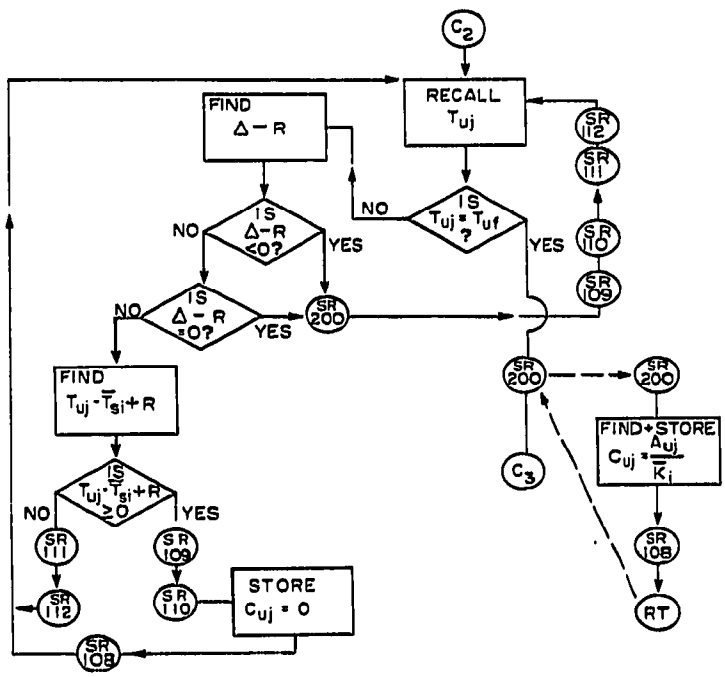

Fig. 3. Flow diagram of data editing and calculations...

dition 1, the unknown peak is considered to be identical with the calibration standard with which it is being compared, and SR 200 causes the PEC to calculate the concentration of the unknown amino acid (C_{uj}) according to eqn. 7:

$$C_{uj} = A_{uj}/\overline{K}_l \tag{7}$$

SR 200 in turn signals SR 108, followed by SR 109–112 and the next comparison. When the last unknown and identifiable peak is reached, SR 200 is performed and the PEC calls upon that part of the program depicted in Fig. 4.

The first two columns in Fig. 4 show the flow diagrams for correcting the concentration of the identified amino acids (C_{cjm}) by means of internal standards according to eqn. 8:

$$C_{cjm} = C_{uj} \cdot C_s/C_{mI} \tag{8}$$

where $m = 1$ or 2, C_{1I} = concentration of β-AGP and C_{2I} = concentration of Nle. Each value of C_{cjm} is operated upon by SR 201 and SR 202 to give values of weight percentage of sample (W_j) and weight percentage of protein (P_j), respectively, for each amino acid as calculated by eqns. 9 and 10:

$$W_j = C_{cjm} \cdot SF \cdot DF \cdot MWT_j \cdot 10^2/\text{dry wt.} \tag{9}$$

where SF converts C_{cjm} into moles/liter, DF is the dilution factor, and MWT_j is the anhydrous molecular weight;

$$P_j = W_j/N \cdot 6.25^* \tag{10}$$

where N is the percentage nitrogen from a Kjeldahl determination. Subroutines 201

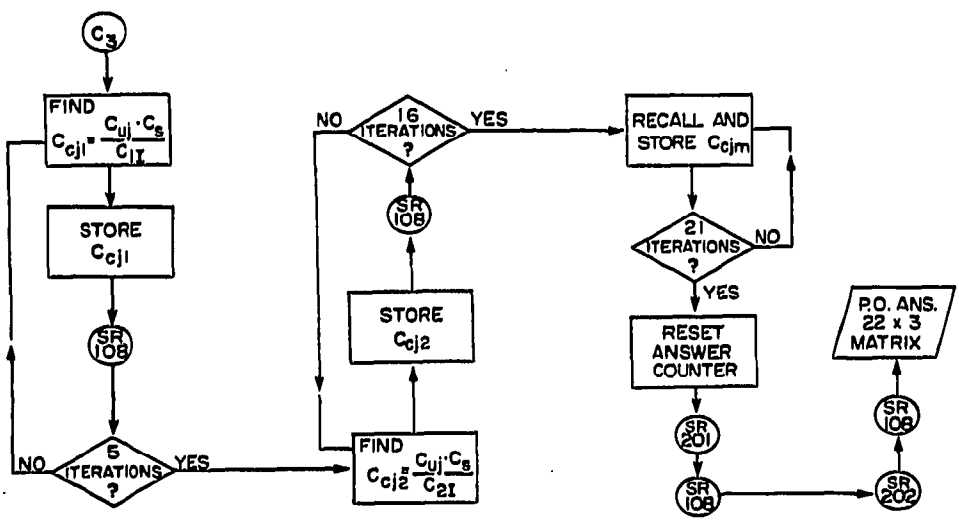

Fig. 4. Flow diagram of calculations and data printout.

* By inserting the appropriate digits into program steps 476, 478 and 479, any three-digit number can be used as a protein factor.

and 202 also calculate ΣW_j and ΣP_j, excluding the values for the internal standards.

In column 4 in Fig. 4, all values including totals are printed out in a 22×3 matrix. A considerable number of programming steps was saved by using an 8×11 in. data sheet, shown in Fig. 5, so that the printer was required only to type numerical values in appropriate spaces. This is possible because the printer is a modified IBM Selectric typewriter capable of accepting standard-sized paper. The numbers shown in Fig. 5 indicate the locations of storage registers.

The last two columns in Fig. 5 give each amino acid as a percentage of the sample dry weight and of crude protein (Kjeldahl). Presentation of the data in this form is an accepted convention among plant breeders, animal nutritionists and others, whose interest lies in evaluating the nutritive worth of proteins in feedstuffs. In these, it is generally accepted that the usefulness of proteins primarily depends upon two factors, the total concentration of crude protein (*i.e.*, nitrogen $\times 6.25$) and the distribution of amino acid constituents in the protein[10]. Moreover, values of interest to others (*e.g.*, molar quantities) can be introduced into the last two columns in Fig. 5 simply by rewriting subroutines SR 201 and SR 202.

Finally, by substituting the total atomic weight of nitrogen in each amino acid (*e.g.*, lysine 28; histidine 42; arginine 56; the remainder 14) for MWT_j which appears in eqn. 9, one can obtain a recovery strictly based on nitrogen and independent of any protein factor.

SAMPLE _____ C _____

	CONCENTRATION µm/ml	% of DRY WT. grams/100 g	% of PROTEIN grams/100 g
Lys	83	84	85
His	86	87	88
NH_3	89	90	91
a-AGP	92	93	94
Arg	95	96	97
Cys A	98	99	100
Asp	101	102	103
Thr	104	105	106
Ser	107	108	109
Glu	110	111	112
Pro	113	114	115
Gly	116	117	118
Ala	119	120	121
Cys	122	123	124
Val	125	126	127
Met	128	129	130
Ile	131	132	133
Leu	134	135	136
Nle	137	138	139
Tyr	140	141	142
Phe	143	144	145
TOTALS		16	17

STANDARDS

C _____ C _____

RANGE 00 _____

DRY WT. 01 _____

% N 02 _____

DILUTION 03 _____

Fig. 5. Sample data sheet containing storage locations of data.

OPERATING PROCEDURE

1. Load program tape; depress REWIND, TAPE READY, RUN, key PRIME, LOAD PROGRAM, VERIFY PROGRAM; read 6217 in X Register; key PRIME.
2. Key GO.
3. Index range.
4. Key GO.
5. Index dry weight in grams.
6. Key GO.
7. Index percentage nitrogen (Kjeldahl).
8. Key GO.
9. Index dilution volume.
10. Key GO.
11. Insert punch tape of molecular weights of calibration standards in chronological order of elution.
12. Key GO.
13. Insert tape containing first set of calibration data. Use tape reader on teletypewriter to delete any extraneous peaks on both standard tapes.
14. Key GO.
15. Insert tape containing second set of calibration data.
16. Key GO.
17. Insert tape containing unknown data.
18. Key GO.
19. Insert data sheet in typewriter.
20. Key PRIME, SEARCH.
21. Set toggle switches to 806.
22. Key GO.

To obtain a new analysis using standards already in core memory:

1. Key PRIME.
2. Repeat steps 2–10 as above.
3. Insert tape containing unknown data.
4. Key PRIME, SEARCH, CHANGE SIGN, GO.
5. Key PRIME, SEARCH.
6. Set toggle switches to 806.
7. Key GO.

To obtain a second printout of answers, key SEARCH, GO.

APPENDIX PROGRAM Title: Amino Acid No. 6217

STEP	CODE	KEY
0000	04 08	MARK
0001	07 00	700
0002	07 01	1
0003	07 11	CHANGE SIGN
0004	06 04	†
0005	04 08	MARK
0006	07 10	710
0007	07 01	1
0008	06 00	+
0009	05 15	STOP
0010	05 04	STORE INDIR
0011	07 03	3
0012	05 09	SKIP IF Y=X
0013	04 07	SEARCH
0014	07 10	SET EXP
0015	04 08	MARK
0016	08 11	811
0017	05 15	STOP
0018	04 09	GROUP 1
0019	00 07	0007
0020	07 01	1
0021	07 09	9
0022	06 04	†
0023	04 08	MARK
0024	07 01	701
0025	07 01	1
0026	06 00	+
0027	04 09	GROUP 1
0028	00 00	0000
0029	05 04	STORE INDIR
0030	07 04	4
0031	07 00	0
0032	05 09	SKIP IF Y=X
0033	04 07	SEARCH
0034	07 01	1
0035	04 08	MARK
0036	07 02	702
0037	05 15	STOP
0038	04 09	GROUP 1
0039	00 07	0007
0040	07 04	4
0041	07 00	0
0042	06 04	†
0043	04 08	MARK
0044	07 04	704
0045	07 01	1
0046	06 00	+
0047	04 09	GROUP 1
0048	00 00	0000
0049	05 04	STORE INDIR
0050	07 08	8
0051	07 02	2
0052	05 09	SKIP IF Y=X
0053	04 07	SEARCH
0054	07 04	4
0055	04 08	MARK
0056	07 05	705
0057	05 15	STOP
0058	04 09	GROUP 1
0059	00 07	0007
0060	07 04	4
0061	07 00	0
0062	06 04	†
0063	04 08	MARK
0064	07 06	706
0065	07 01	1
0066	06 00	+
0067	04 09	GROUP 1
0068	00 00	0000
0069	05 00	+ INDIR
0070	07 08	8
0071	07 02	2
0072	05 09	SKIP IF Y=X
0073	04 07	SEARCH
0074	07 06	6
0075	04 08	MARK
0076	07 07	707
0077	07 03	3
0078	07 09	9
0079	06 04	†
0080	04 08	MARK
0081	07 08	708
0082	07 02	2
0083	06 00	+
0084	05 03	÷ INDIR
0085	07 08	8
0086	07 01	1
0087	05 09	SKIP IF Y=X
0088	04 07	SEARCH
0089	07 08	8
0090	04 08	MARK
0091	07 09	709
0092	07 04	4
0093	07 00	0
0094	06 04	†
0095	04 08	MARK
0096	07 11	711
0097	07 08	8
0098	07 02	2
0099	06 04	†
0100	05 15	STOP

STEP	CODE	KEY
0101	04 09	GROUP 1
0102	00 07	0007
0103	04 08	MARK
0104	07 12	712
0105	07 01	1
0106	06 00	+
0107	04 09	GROUP 1
0108	00 00	0000
0109	05 04	STORE INDIR
0110	07 15	CL X
0111	04 07	SEARCH
0112	07 12	DEC POINT
0113	04 08	MARK
0114	08 06	806
0115	04 04	STORE DIR
0116	01 06	REG 16
0117	04 04	STORE DIR
0118	01 07	REG 17
0119	07 02	2
0120	07 00	0
0121	04 04	STORE DIR
0122	00 07	REG 07
0123	07 08	8
0124	07 03	3
0125	04 04	STORE DIR
0126	00 08	REG 08
0127	07 04	4
0128	07 01	1
0129	04 04	STORE DIR
0130	00 09	REG 09
0131	07 04	4
0132	07 02	2
0133	04 04	STORE DIR
0134	00 10	REG 10
0135	07 08	8
0136	07 03	3
0137	04 04	STORE DIR
0138	00 11	REG 11
0139	07 08	8
0140	07 04	4
0141	04 04	STORE DIR
0142	00 12	REG 12
0143	04 08	MARK
0144	07 13	713
0145	04 15	RE DIR (Y)
0146	00 11	REG 11
0147	05 05	RE INDIR
0148	06 04	↑
0149	06 04	↑
0150	04 05	RE DIR(X)
0151	00 05	REG 05
0152	05 09	SKIP IF Y=X
0153	04 07	SEARCH
0154	07 14	RE RES
0155	04 07	SEARCH
0156	07 15	CL X
0157	04 08	MARK
0158	07 14	714
0159	07 15	CL X
0160	06 04	↑
0161	04 15	RE DIR (Y)
0162	00 11	REG 11
0163	05 05	RE INDIR
0164	04 04	STORE DIR
0165	00 04	REG 04
0166	04 15	RE DIR (Y)
0167	00 09	REG 09
0168	05 05	RE INDIR
0169	04 15	RE DIR (Y)
0170	00 11	REG 11
0171	05 01	- INDIR
0172	05 05	RE INDIR
0173	05 04	STORE INDIR
0174	04 04	STORE DIR
0175	00 13	REG 13
0176	06 07	|x|
0177	06 04	↑
0178	04 05	RE DIR (X)
0179	00 00	REG 00
0180	06 01	-
0181	04 12	WRITE α
0182	05 10	SKIP IF Y < 0
0183	04 07	SEARCH
0184	08 00	800
0185	04 08	MARK
0186	08 05	805
0187	02 00	SR 0200
0188	05 14	GO
0189	01 09	SR 0109
0190	01 10	SR 0110
0191	01 11	SR 0111
0192	01 12	SR 0112
0193	04 07	SEARCH
0194	07 13	x^2
0195	04 08	MARK
0196	08 00	800
0197	04 12	WRITE α
0198	04 11	SKIP IF Y=0
0199	04 07	SEARCH
0200	08 07	807

STEP	CODE	KEY
0201	04 07	SEARCH
0202	08 05	805
0203	04 08	MARK
0204	08 07	807
0205	04 05	RE DIR (X)
0206	00 13	REG 13
0207	04 15	RE DIR (Y)
0208	00 00	REG 00
0209	06 00	+
0210	07 00	0
0211	05 07	SKIP IF Y $\geq$ X
0212	04 07	SEARCH
0213	08 01	801
0214	04 07	SEARCH
0215	08 02	802
0216	04 08	MARK
0217	08 01	801
0218	01 11	SR 0111
0219	01 12	SR 0112
0220	04 07	SEARCH
0221	07 13	x^2
0222	04 08	MARK
0223	08 02	802
0224	01 09	SR 0109
0225	01 10	SR 0110
0226	04 15	RE DIR (Y)
0227	00 08	REG 08
0228	07 00	0
0229	05 04	STORE INDIR
0230	01 08	SR 108
0231	04 05	RE DIR (X)
0232	00 04	REG 04
0233	04 15	RE DIR (Y)
0234	00 11	REG 11
0235	05 04	STORE INDIR
0236	04 07	SEARCH
0237	07 13	x^2
0238	04 08	MARK
0239	07 15	715
0240	02 00	SR 0200
0241	04 08	MARK
0242	05 00	500
0243	04 15	RE DIR (Y)
0244	08 06	REG 86
0245	07 02	2
0246	06 02	x
0247	04 14	STORE Y
0248	00 12	REG 12
0249	04 15	RE DIR (Y)
0250	10 01	REG 101
0251	06 02	x
0252	04 14	STORE Y
0253	00 13	REG 13
0254	07 08	8
0255	07 02	2
0256	06 04	↑
0257	04 08	MARK
0258	05 01	501
0259	07 01	1
0260	06 00	+
0261	04 05	RE DIR (X)
0262	00 12	REG 12
0263	05 03	÷ INDIR
0264	07 08	8
0265	07 07	7
0266	05 09	SKIP IF Y=X
0267	04 07	SEARCH
0268	05 01	- INDIR
0269	04 08	MARK
0270	05 02	502
0271	07 01	1
0272	06 00	+
0273	04 05	RE DIR (X)
0274	00 13	REG 13
0275	05 03	÷ INDIR
0276	07 01	1
0277	07 00	0
0278	07 03	3
0279	05 09	SKIP IF Y=X
0280	04 07	SEARCH
0281	05 02	x INDIR
0282	04 08	MARK
0283	05 03	503
0284	07 01	1
0285	07 04	4
0286	07 03	3
0287	04 04	STORE DIR
0288	00 15	REG 15
0289	07 01	1
0290	07 00	0
0291	07 04	4
0292	06 04	↑
0293	04 08	MARK
0294	05 04	504
0295	07 01	1
0296	06 01	-
0297	05 05	RE INDIR
0298	04 14	STORE Y
0299	00 14	REG 14
0300	04 15	RE DIR (Y)

STEP	CODE	KEY
0301	00 15	REG 15
0302	05 04	STORE INDIR
0303	07 03	3
0304	06 01	-
0305	04 14	STORE Y
0306	00 15	REG 15
0307	04 15	RE DIR (Y)
0308	00 14	REG 14
0309	07 08	8
0310	07 03	3
0311	05 09	SKIP IF Y=X
0312	04 07	SEARCH
0313	05 04	STORE INDIR
0314	04 04	STORE DIR
0315	00 08	REG 08
0316	04 08	MARK
0317	05 05	505
0318	04 15	RE DIR (Y)
0319	00 08	REG 08
0320	05 05	RE INDIR
0321	04 04	STORE DIR
0322	00 14	REG 14
0323	07 01	1
0324	06 00	+
0325	04 14	STORE Y
0326	00 08	REG 08
0327	02 01	SR 0201
0328	01 07	REG 17
0329	02 02	SR 0202
0330	01 08	REG 18
0331	04 15	RE DIR (Y)
0332	00 08	REG 08
0333	07 01	1
0334	07 04	4
0335	07 06	6
0336	05 09	SKIP IF Y=X
0337	04 07	SEARCH
0338	05 05	RE INDIR
0339	02 03	SR 0203
0340	05 15	STOP
0341	04 08	MARK
0342	05 14	514
0343	07 08	8
0344	07 06	6
0345	04 04	STORE DIR
0346	01 08	REG 18
0347	04 12	WRITE α
0348	12 00	POWER ON
0349	01 08	CR/LF
0350	00 11	CLEAR TABS
0351	04 13	END α
0352	07 08	8
0353	07 03	3
0354	06 04	↑
0355	04 08	MARK
0356	08 04	804
0357	05 05	RE INDIR
0358	04 11	WRITE
0359	09 04	REG 94
0360	07 01	1
0361	06 00	+
0362	04 05	RE DIR (X)
0363	01 08	REG 18
0364	05 08	SKIP IF Y < X
0365	01 13	SR 0113
0366	05 14	GO
0367	04 05	RE DIR (X)
0368	00 08	REG 08
0369	05 09	SKIP IF Y=X
0370	04 07	SEARCH
0371	08 04	804
0372	04 05	RE DIR (X)
0373	01 06	REG 16
0374	04 11	WRITE
0375	15 15	1515
0376	04 11	WRITE
0377	09 03	93
0378	04 05	RE DIR (X)
0379	01 07	REG 17
0380	04 11	WRITE
0381	09 03	93
0382	07 15	CL X
0383	06 04	↑
0384	05 15	STOP
0385	04 08	MARK
0386	01 07	107
0387	07 07	7
0388	06 04	↑
0389	07 01	1
0390	05 00	+ INDIR
0391	05 11	RETURN
0392	04 08	MARK
0393	01 08	108
0394	07 08	8
0395	06 04	↑
0396	07 01	1
0397	05 00	+ INDIR
0398	05 11	RETURN
0399	04 08	MARK
0400	01 09	109

STEP	CODE	KEY
0401	07 09	9
0402	06 04	↑
0403	07 02	2
0404	05 00	+ INDIR
0405	05 11	RETURN
0406	04 08	MARK
0407	01 10	110
0408	07 01	1
0409	07 00	0
0410	06 04	↑
0411	07 02	2
0412	05 00	+ INDIR
0413	05 11	RETURN
0414	04 08	MARK
0415	01 11	111
0416	07 01	1
0417	07 01	1
0418	06 04	↑
0419	07 02	2
0420	05 00	+ INDIR
0421	05 11	RETURN
0422	04 08	MARK
0423	01 12	112
0424	07 01	1
0425	07 02	2
0426	06 04	↑
0427	07 02	2
0428	05 00	+ INDIR
0429	05 11	RETURN
0430	04 08	MARK
0431	02 00	200
0432	04 15	RE DIR (Y)
0433	00 10	REG 10
0434	05 05	RE INDIR
0435	04 15	RE DIR (Y)
0436	00 12	REG 12
0437	05 03	÷ INDIR
0438	05 05	RE INDIR
0439	04 15	RE DIR (Y)
0440	00 08	REG 08
0441	05 04	STORE INDIR
0442	01 08	SR 108
0443	05 11	RETURN
0444	04 08	MARK
0445	02 01	201
0446	04 05	RE DIR (X)
0447	00 03	REG 03
0448	04 02	x DIR
0449	00 14	REG 14
0450	04 05	RE DIR (X)
0451	00 01	REG 01
0452	04 03	÷ DIR
0453	01 04	REG 14
0454	04 15	RE DIR (Y)
0455	00 07	REG 07
0456	05 05	RE IND
0457	04 12	WRITE α
0458	04 04	STORE DIR
0459	04 02	x DIR
0460	00 14	REG 14
0461	04 05	RE DIR (X)
0462	00 14	REG 14
0463	04 15	RE DIR (Y)
0464	00 08	REG 08
0465	05 04	STORE INDIR
0466	04 00	+ DIR
0467	01 06	REG 16
0468	01 08	SR 0108
0469	05 11	RETURN
0470	04 08	MARK
0471	02 02	202
0472	04 05	RE DIR (X)
0473	00 02	REG 02
0474	04 03	÷ DIR
0475	00 14	REG 14
0476	07 06	6
0477	07 12	.
0478	07 02	2
0479	07 05	5
0480	04 03	÷ DIR
0481	00 14	REG 14
0482	04 05	RE DIR (X)
0483	00 14	REG 14
0484	04 12	WRITE α
0485	07 02	702
0486	04 15	RE DIR (Y)
0487	00 08	REG 08
0488	05 04	STORE IND
0489	04 00	+ DIR
0490	01 07	REG 17
0491	05 11	RETURN
0492	04 08	MARK
0493	00 00	0000
0494	04 08	MARK
0495	08 08	808
0496	07 02	2
0497	06 01	-
0498	06 05	↓
0499	04 04	STORE DIR
0500	00 06	REG 06

STEP	CODE	KEY
0501	05 05	RE INDIR
0502	04 04	STORE DIR
0503	00 05	REG 05
0504	04 15	RE DIR (Y)
0505	08 01	REG 81
0506	05 08	SKIP IF Y < X
0507	04 07	SEARCH
0508	08 09	809
0509	06 01	-
0510	06 05	↓
0511	06 07	| x |
0512	04 15	RE DIR (Y)
0513	00 00	REG 00
0514	05 07	SKIP IF Y ≥ X
0515	04 07	SEARCH
0516	08 10	810
0517	04 07	SEARCH
0518	08 09	809
0519	04 08	MARK
0520	08 10	810
0521	04 15	RE DIR (Y)
0522	00 06	REG 06
0523	04 07	SEARCH
0524	08 08	808
0525	04 08	MARK
0526	08 09	809
0527	05 11	RETURN
0528	04 08	MARK
0529	01 13	113
0530	07 03	3
0531	04 00	+ DIR
0532	01 08	REG 18
0533	04 12	WRITE α
0534	01 08	CR/LF
0535	01 10	LF (INDEX)
0536	04 13	END α
0537	05 11	RETURN
0538	04 08	MARK
0539	02 03	203
0540	04 15	RE DIR (Y)
0541	09 03	REG 93
0542	04 05	RE DIR (X)
0543	13 08	REG 138
0544	06 00	+
0545	06 05	↓
0546	04 01	- DIR
0547	01 06	REG 16
0548	04 15	RE DIR (Y)
0549	09 04	REG 94
0550	04 05	RE DIR (X)
0551	13 09	REG 139
0552	06 00	+
0553	06 05	↓
0554	04 01	- DIR
0555	01 07	REG 17
0556	05 11	RETURN
0557	05 12	END PROG

REFERENCES

1 D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, 30 (1958) 1190.
2 K. A. Piez and L. Morris, *Anal. Biochem.*, 1 (1960) 197.
3 P. B. Hamilton, *Anal. Chem.*, 35 (1963) 2055.
4 A. S. Inglis, *Anal. Biochem.*, 7 (1964) 168.
5 W. L. Porter and E. A. Talley, *Anal. Chem.*, 35 (1964) 1692.
6 M. I. Krichevsky, J. Schwartz and M. Mage, *Anal. Biochem.*, 12 (1965) 94.
7 H. L. Back, P. J. Buttery and K. Gregson, *J. Chromatogr.*, 68 (1972) 103.
8 H. D. Spitz, G. Henyon and J. N. Sivertson, *J. Chromatogr.*, 68 (1972) 111.
9 *Automatic Amino Acid Analyzer, Instruction Manual*, Spinco Division of Beckman Instruments Inc., Palo Alto, Calif., p. 4-31.
10 E. W. Crompton and L. E. Harris, *Applied Animal Nutrition*, W. H. Freeman and Co., San Francisco, 2nd ed., 1969, Ch. 4, p. 88.