in an alkaline Polycell solution for at least 24 h before analysis. When these steps are taken blockage of the manifold occurs infrequently.

N excretion in man is predominantly urinary; about 10% of the intake is excreted in the faeces. The main source of error in a N balance study is in the estimation of dietary N. Because of the mixed nature of the diet it is difficult to obtain a truly homogeneous sample and to draw from it representative portions. This difficulty can be overcome to a great extent by mincing all meat and vegetables, by using an efficient homogenizer, and sampling by siphon while mixing.

Values for N balance have been obtained for many patients in the Metabolic Unit. Large numbers of samples are required and these must be analysed under comparable conditions. We have found that the Technicon AutoAnalyzer provides an accurate and reproducible alternative to the manual Kjeldahl technique.

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Problems associated with the automated analysis of mixtures of amino acids

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Many nutritional investigations now in progress require a knowledge of amino acid concentrations in the diet and in tissues so that the requirements of the animal under study for each amino acid may be estimated, and adequacy of dicts assessed. Analysis for amino acids was difficult and slow before the advent some two decades ago of methods using ion exchange resins for separating all the amino acids in a mixture by column chromatography. The original method of Moore & Stein (1951) taking about 1 week per analysis was developed (Moore & Stein, 1954*a*,*b*; Moore, Spackman & Stein, 1958) and then automated (Spackman, Stein & Moore, 1958) until one complete analysis could be achieved each day.

More rapid analysis has been possible following (1) the introduction of a spherical form of resin which can be graded accurately for size according to methods such as that of Hamilton (1958) thus allowing more uniform packing of columns and (2) the development of pumps reliable at high pressures (up to 1000 lb/in^2) and low volume deliveries (3–120 ml/h).

At the Rowett Institute the advent of rapid and more precise chromatographic methods gave rise to an increasing demand for amino acid analysis and the single column system purchased from the Technicon Instruments Company, Chertsey, Surrey, allowing one analysis/d was soon unable to satisfy the demand for analyses. Increased output could be achieved either by replicating the whole or part of the system or by decreasing the time of each analysis from 20 h to about 5 or 6 h and arranging to have an automatic procedure for the necessary loading, development and regeneration during periods when the column was unattended

We decided to test a system based on the latter highly automated procedure because, apart from the question of expense in replicating systems, it was considered advantageous to have only one column with a single set of characteristics rather than several, each with characteristics liable to be different because of factors such as variation in batches of resin and packing characteristics in the column. In addition, it was planned to make use of the I.B.M. 1130 Computer (International Business Machines, London) sited at the Rowett Institute for calculation of results. It is our experience in the development of this system that I wish now to describe.

The column and running conditions

The single column used for amino acid analysis in 20 h measured $140 \times 0{\cdot}6$ cm diameter and was filled with cation exchange bead resin (Technicon Chromobeads B) which had diameters mainly 17 ± 3 μm. Pump pressures required for completion of an analysis in even 9 h on this column increased from the 300–350 lb/in^2 to which we were accustomed in the 20 h runs to over 800 lb/in^2 with a tendency for the pump to leak. It was evident that, with the Milton-Roy Mk I pump which we were using, either the length of the column would have to be reduced or the particle size of the resin would have to be increased. We decided to retain the resin and decrease column length to about $70 \times 0{\cdot}6$ cm. A run could now be completed in 6 h at 300–400 lb/in^2.

After some preliminary work based on our experience of conditions suitable for the long column, the separations we could achieve on this short column were generally satisfactory but tended to be poor for Glu/Pro and Tyr/Phe. Raising the column temperature from 60 to 66° allowed us to separate Pro from Glu and further small alterations in the buffer components of the Autograd allowed a separation in which peaks on the chromatogram from a standard mixture of seventeen amino acids were sufficiently differentiated to allow calculation by the triangulation method although on occasion Tyr and Phe were still not adequately separated. The short fast-running column now seemed suitable for protein hydrolysates, but it did not prove adequate for complete separation of the extra amino acids in plasma and tissue fluids.

Automatic loading and control

The next step was to fit automatic loading and control devices for overnight running. Eveleigh & Thomson (1966) and Thomson (1967) described new devices for automatic loading of samples as well as for control of the development of the chromatogram and cleaning and regeneration of the column. These devices, which seemed suitable for our requirements were obtained through Dr. A. R. Thomson from the Wantage Research Laboratories of the United Kingdom Atomic Energy Authority. (Equipment based on such principles is now being manufactured under licence and is obtainable from the Technicon Instruments Company Ltd, Chertsey, Surrey, and Evans Electroselenium Ltd, Halstead, Essex.)

Sample loader. That designed by Eveleigh & Thomson (1966) consists of a Perspex block holding nylon tubes each containing a small amount of resin which

can be considered as a small extension of the resin in the column but separated from that resin by a fine column of buffer. By means of slots in the sides, this block can be moved forward one position on command by a motorized Geneva wheel so that each tube is brought in turn into the line of the developing solvent which is pumped through at about 57 ml/h by a Milton-Roy pump.

Multi-channel programming valve. The particular solvent pumped through the column is determined by a nine-channel regulating valve (Eveleigh & Thomson, 1966) with twelve positions operating on the principle of a peristaltic pump with discontinuous operation. This valve can be set so that the desired buffer, cleaning and regenerating fluids for each analysis cycle are available at predetermined times for pumping through the column.

Timing device. These times are determined by a device in which pegs on a turn-table mounted on a synchronous motor make contact at pre-set times with micro-switches. Through a system of relays these switches activate the motors of the sample loader and programming valve.

A more convenient timer might be one working on an endless belt principle whereby a loop of plastic perforated at the desired points is driven by a synchronous motor past one or more micro-switches the latter being actuated as the arms drop through the perforations. The advantage in this case is that the loop can be made to any size and reproduces accurately a given sequence of commands.

Sampling the colorimeter output by a data logger with punched tape output suitable for the IBM 1130 Computer

The continuous output from each Technicon colorimeter of the amino acid analyser is in the form of small out-of-balance voltages from paired photocells one of which continuously monitors the colour intensity in the flowing liquid stream representing the eluate from the bottom of the chromatographic column. Normal output on the recorder chart of the analyser is in the form of a plot of out-of-balance voltages against time in which the emergence of amino acids from the column is recorded as a series of peaks. Peak areas have to be calculated and related to amino acid concentration by the use of appropriate factors obtained from the analyses of standard mixtures.

In modifying this system to obtain an output on punched paper tape suitable for direct acceptance by the computer the following points were considered.

Sampling points

There were several ways of sampling the changing voltage from the colorimeters. One was to use the recorder itself and by means of devices such as a retransmitting slidewire or optical shaft encoder working mechanically in parallel to the recorder slidewire, to take readings at time intervals dictated by the existing recorder system. In our Technicon recorder this was about 7 sec. However, reading at such intervals or multiples thereof may not always be suitable. For example we may wish to run very fast chromatograms taking 3 or less h and wish to sample at 3 or 4 sec intervals whereas for a 20 h chromatogram in which amino acid separation has been found

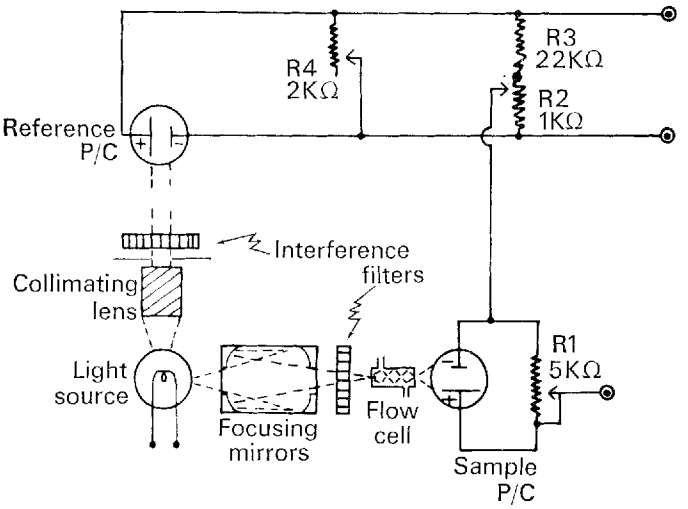

Fig. 1. Layout of Technicon colorimeter showing position of additional resistance R4.

difficult an interval between 10 and 30 sec may be adequate and therefore desirable to reduce tape output.

An alternative to sampling from the recorder was to feed the signal directly from one or more of the three colorimeters into the data-logging system using manually variable intervals chosen to suit the rate of running of the chromatogram. Besides greater flexibility in sampling interval, this would have the additional advantage that recorder and data-logger operation would be independent, and in the event of either developing a fault either a chart-recorder trace or tape record would still be available for examination.

We therefore decided to sample directly from two of the three colorimeters for the data-logging system and to retain output from only one colorimeter for a visual representation on the chart recorder.

The colorimeters

The layout of the Technicon colorimeter shown in modified form in Fig. 1 has 'sample' and 'reference' photocells of the selenium barrier-layer type wired in opposition so that a difference in voltage (out-of-balance voltage) is obtained when light to the sample photocell is reduced owing to absorption by the test liquid. The optical screening of light and the flowcell in front of the sample photocell is different from the optical arrangement in front of the reference cell. The resultant difference in voltage output from the photocells can be adjusted electrically to zero by variable resistances placed across each photocell. In the arrangement as supplied, the voltage emanating from the reference cell is inherently greater than that from the sample cell. The output across the reference cell is about 170 mV under the conditions used for measuring the blue colour formed between ninhydrin and amino acids, whereas across the sample photocell it is between 2 and 30 mV depending on the setting of the 5000 Ω resistor. It was considered desirable to retain the balancing system

normally used in these colorimeters because there would be some compensation for any variation in intensity of energy from the light source.

Balancing output from the photocells. Output from the photocells can be brought to balance by introducing an additional variable shunt across the reference photocell or by reducing the light falling on the reference photocell by placing small apertures or neutral filters between light source and photocell. It was found, however, that the aperture required to obtain balance under normal control conditions was so small that it was considered undesirable on the grounds that only a very small part of the reference photocell would be activated by the transmitted light and this might lead to undesirable fatigue effects which would be magnified by any small displacement of the aperture or the photocell. Optical balancing with a neutral filter (Chance ON 32, 38% transmission) between the interference filter and reference photocell seemed no more stable than electrical balancing. This electrical method was therefore chosen because of its greater convenience.

A variable shunt R4 in Fig. 1, of about 2000 Ω was inserted across the reference photocells and the isolated output adjusted to 20 mV. The difference in output voltage between sample and reference photocells was then adjusted to zero by the 5000 Ω shunt across the sample photocell. When light to the latter was cut off completely (infinity optical density of test solution) the full-scale out-of-balance voltage of 20 mV was registered.

Preliminary test. With various concentrations of norleucine in solution we obtained out-of-balance voltage readings on tape. According to the Beer-Lambert equation $C=k \log_{10} \frac{1}{\text{transmittance}}$, where C=concentration of amino acids in g/l, k=a constant, and transmittance = ratio of incident to transmitted light. In our case

$$C=k \log_{10} \left(\frac{20}{20-X}\right), \quad (1)$$

where X is the mV reading corresponding to light absorbed by the test solution in the flowcell and 20 is the voltage reading in mV corresponding to the pre-set intensity of the incident light as indicated by the reference photocell.

An essentially linear relationship was obtained between norleucine concentration and $\log \frac{20}{20-X}$ readings thus indicating that a normal Beer-Lambert relationship existed for our modified colorimeter system.

The paper tape

The interval chosen for sampling each colorimeter output depends on several factors. It is desirable for accuracy to have at least twenty readings for each colour peak in the flowing stream and, if the duration of the smallest peak is about 4 min, as in a 5 h chromatogram, then a sampling interval around 12 sec is advisable. Our Solartron data logger can scan at intervals of 2, 5, 10, 20 and 60 sec and so if both 440 and 570 nm colorimeters are used, alternate scanning at a 5 sec interval is indicated. This gives sampling of each colorimeter voltage every 10 sec.

At first each reading was recorded as a single digit channel number, polarity, a 4 digit voltage reading and an end-of-reading code, i.e. seven rows of punched holes on the tape occupying 0·7 in tape. At a 5 sec interval, in 20 h the 14 400 readings would occupy 10 080 in or 840 ft of tape, a length well within the 1000 ft available. In order to keep tape output to a minimum, it was decided to omit polarity indication and ensure that all readings were positive. This was achieved by setting the reference photocell output to 21·00 instead of 20·00 mV and then the out-of-balance voltage to 1·00 mV. Equation 1 then becomes

$$C = k \log_{10} \left(\frac{20}{21 - X} \right). \quad (2)$$

A further saving in tape can be made between analyses when the column is being cleaned and regenerated. The most convenient method of doing this is to interrupt the signal from the digital voltmeter to the punch drive unit by a cam device on the 24 h turntable timer acting through a micro-switch which also causes an end-of-analysis code to be punched on tape. A micro-switch working in parallel, actuated by the same cam, interrupts the recorder drive unit so that, by reference to the visual recording, any peak can be located on tape.

Programming for the computer

In collaboration with Mr A. W. Boyne of the Biometry Department at the Rowett Research Institute, a programme has been devised to read the paper tape output from the data logger and to ascertain that the scanner has controlled the sequence of punching correctly, i.e. that the logger channels are recorded in correct sequence. Values which are obviously incorrect are replaced by mean values worked out from adjacent data.

After the data have been accepted by the computer a smoothing process is carried out and subsequent analysis referred to the smoothed data. Although, with somewhat better separation and more consistent peak parameters than was possible on the 5·5 h chromatograms, other methods may provide acceptable estimates, the most recent method of estimating areas has been to multiply width at half height by maximum height for each component. It is possible to estimate areas on the assumption that neighbouring peaks have similar shape characteristics and then to estimate parameters for overlapping peaks on an iterative basis.

The particular amino acids present are identified from an examination of the visual record and preparation of a punched card for the computer, to indicate where each amino acid is located on the tape. The programme refers to this information supplied by card and then extracts the appropriate colour factor from a file stored on disk thus enabling estimates to be made of each amino acid. A printout can be given in any one or all of several forms.

A typical output is given in Table 1 for an insulin hydrolysate. This table shows the time of occurrence of each amino acid after the start of the chromatogram, the micromoles in the sample, the percentage of amino acid nitrogen in the total sample nitrogen, the molar proportions of each amino acid estimated and the absolute amount of each amino acid in 16 g total nitrogen.

Table 1. *Typical printout of amino acid results from the computer with a comparative set of figures in g/16 g nitrogen worked out from a recorder chart run in parallel : insulin hydrolysate*

	Computer printout					Calculated from recorder chart (g/16 g N)
	Time after start (min)	μmoles	Recovery of nitrogen (%)	Molar proportions	g/16 g N	
Cys $\bar{A}$	3	0·045	0·73	1·03	1·42	1·44
Asp	41	0·274	4·44	6·27	6·75	6·76
Thr	49	0·096	1·55	2·19	2·11	2·36
Ser	53	0·265	4·29	6·05	5·15	5·18
Glu	65	0·738	11·95	16·87	20·09	18·88
Gly	92	0·398	6·45	9·11	5·53	5·49
Ala	98	0·292	4·72	6·67	4·81	4·79
Val	119	0·358	5·80	8·19	7·76	7·72
CyS	130	0·380	6·15	8·69	8·45	8·50
Ile	155	0·037	0·59	0·84	0·89	0·97
Leu	160	0·537	8·69	12·27	13·03	12·85
Tyr	177	0·325	5·26	7·42	10·89	9·50
Phe	182	0·248	4·01	5·66	7·57	7·19
Lys	255	0·104	3·36	2·37	2·81	2·82
His	267	0·175	8·48	3·99	5·01	5·06
Arg	310	0·101	6·57	2·32	3·27	3·16

The last column in the table giving the concentration of each amino acid calculated by the manual method from a corresponding chart output indicates that this programme works well with chromatograms where separation is reasonably well defined.

Sources of error

In trying to reduce variability to a minimum we investigated several sources of error.

Errors arising at the colorimeter

In the arrangement which we have been using, only the out-of-balance voltage has been continuously monitored on tape. We assume that the preset photocell output value of 20 mV, representing the incident light, remains constant during the run overnight. Fig 2. shows that the linearity of response originally obtained between amino acid concentration and calculated optical density was improved by taking into consideration fluctuations in light intensity from the colorimeter lamp. By placing a neutral filter in front of the sample photocell and continuously monitoring output from the reference cell as well as out-of-balance voltage we found that variation in calculated optical density values as great as 3% could be reduced to 1% by taking into consideration variation of 2% found in reference cell output. However, continuous recording of reference cell voltage as well as out-of-balance voltage could double the number of readings to be recorded on tape and this is to be avoided if possible. It is essential then to reduce to a minimum all factors which might affect light intensity and photocell response to light intensity. Should the factors involved prove too difficult to control within the limits required then it may be necessary to

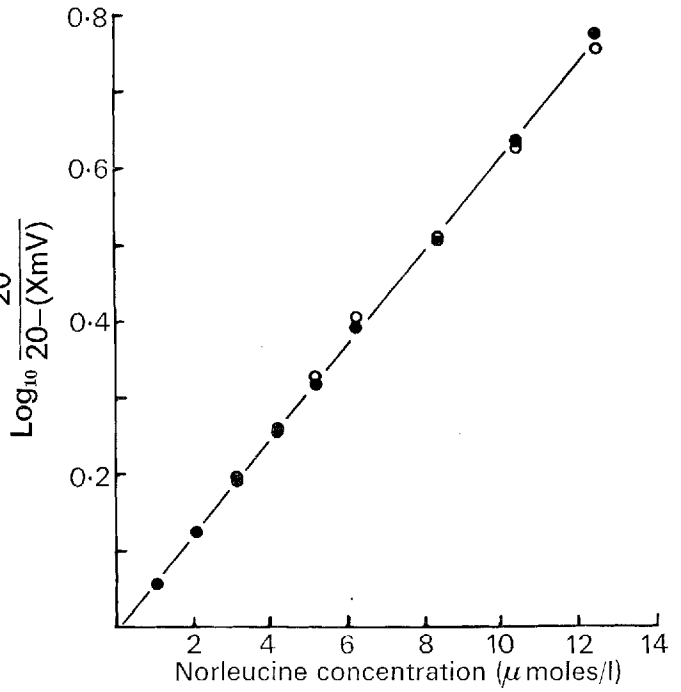

Fig. 2. Relationship between norleucine concentration and colour intensity developed before (○) and after (●) correction for reference photocell deviation from 20 mV.

devise some electronic feed-back system or to use another type of colorimeter incorporating such a system.

Errors arising from variations of energy supplied to the lamp

When considering an unexplained drift of 1–2% in the out-of-balance voltage encountered in an overnight test run with our newly modified colorimeter we used the data-logger to monitor output from the reference cells of two colorimeters and found that maximum and minimum voltages occurred at the same time (Fig. 3) indicating a mains variation effect with a range of about 3%. The voltage stabilizer being used was rated at 6 V r.m.s. ± 1%.

When the stabilizer was replaced by a 6 V accumulator which was simply allowed to discharge through the colorimeter lamp, the percentage fall in voltage output from the reference cell was about thrice that of the voltage supplied to the lamp (Fig. 4). In order to have 1% fluctuation in light intensity it is thus necessary to have 0·3% fluctuation in the voltage to the lamp. A stabilizer having a stabilizing ratio of 1000:1 was found to give a much more steady input with a voltage range of 0·5% or better. We now use a stabilizer having a 10 000:1 stabilizing ratio.

Errors arising from environmental temperature

Fig. 5 shows that even with a high performance voltage stabilizer, over a prolonged

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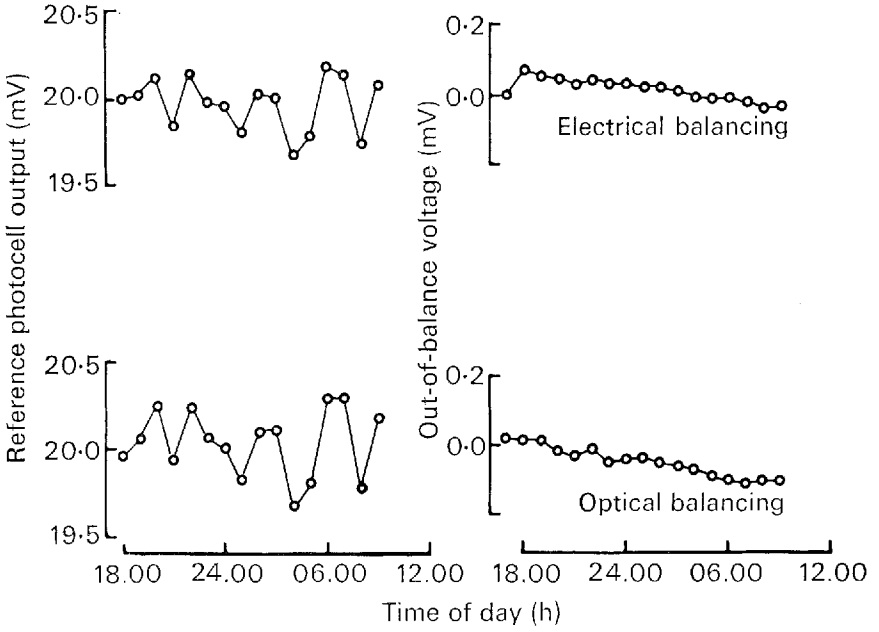

Fig. 3. Effect of variation in input voltage on energy emitted by the colorimeter lamp and the corresponding out-of-balance voltages recorded by electrical and largely optical balancing of photocells.

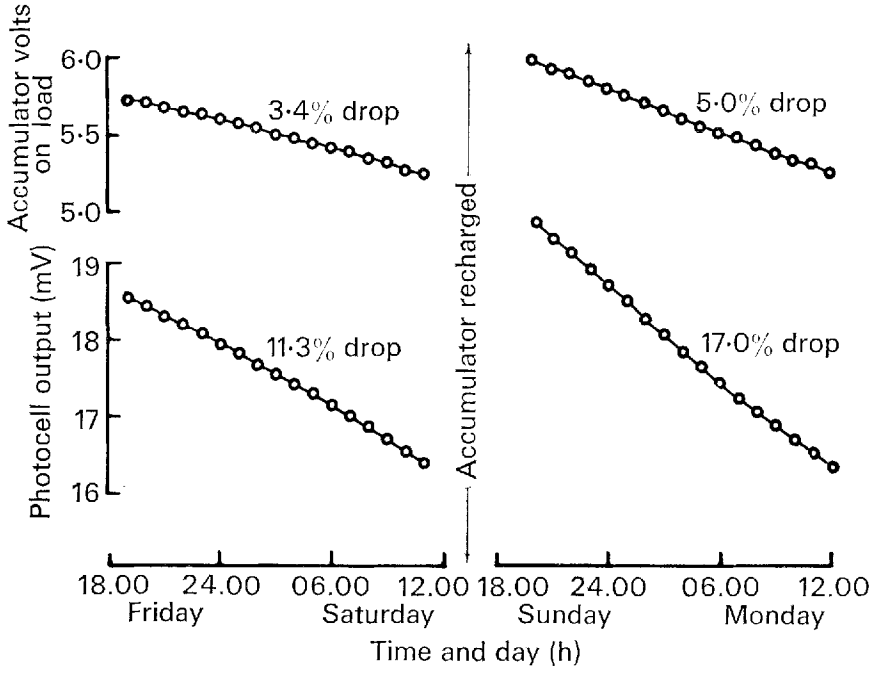

Fig. 4. Effect of variation in energy supplied to the lamp on the resultant energy emitted by the lamp.

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period the reference photocell output may vary by several percent owing to temperature variation in the laboratory. It may thus be necessary to control more closely the environment of the colorimeter housing.

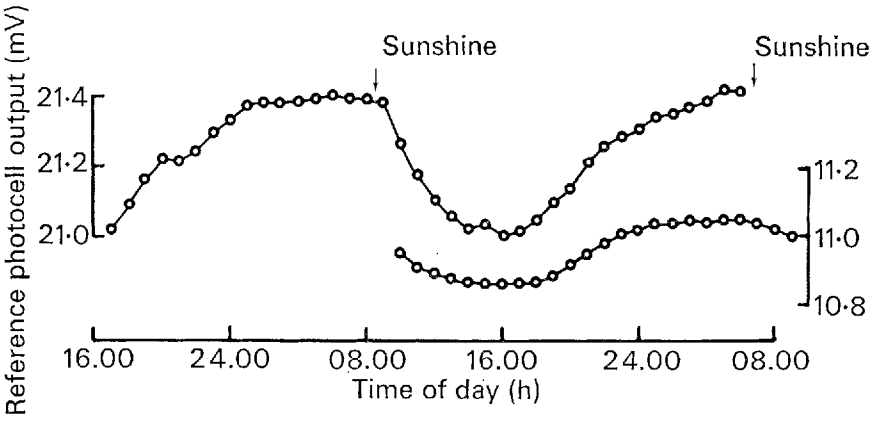

Fig. 5. Effect of environmental temperature on reference photocell output of the colorimeters.

Errors arising at the loader

Each small nylon tube containing resin was originally filled under suction so that variable volumes of hydrolysate could be loaded. However, it was soon noted that there were considerable losses of amino acids such as cysteic acid and taurine, normally expected near the first buffer front. It became obvious that these amino acids were being lost when filling the tube and only a loading system allowing complete retention of sample till placed in the buffer line would be satisfactory. We therefore made new tubes from polytetrafluoroethylene (P.T.F.E.) rod and experimented with different diameters of fine holes in the base to find the largest diameter which would retain samples by capillary forces alone. The size found suitable was 0·1 mm and only three holes were drilled. After further tests it was decided that the safest procedure would be to combine the tube with small holes and the original short column of resin in case there was leakage during loading. Recovery of cysteic acid appeared then to be reasonable. More recently we began to suspect that error was arising from an overall loss of amino acids from the 70 cm column in runs loaded automatically.

We therefore took two hydrolysates of insulin and analysed them, one on a 140 × 0·6 cm column with manual loading and the other on the 70 × 0·6 cm column with both manual and automatic loading followed by automatic development to obtain the values in Table 2.

These show that the estimates of concentration of each amino acid were low on the short column loaded automatically. The percentage of total nitrogen recovered as amino acid nitrogen was around 15% lower by the automatic loading than by manual loading on either the short or the long columns.

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Table 2. *Comparison of estimates of amino acids from 570 nm trace in two hydrolysates of insulin on 140 and 70 cm columns (g/16 g nitrogen)*

Hydrolysis time	24 h			48 h		
Column length (cm)	140	70	70	140	70	70
Analysis time (h)	20	6	6	20	6	6
Type of loading	Manual	Manual	Automatic	Manual	Manual	Automatic
Asp	6·7	6·8	6·3	7·3	7·0	5·7
Thr	2·1	2·4*	1·9*	2·2	2·3*	1·5*
Ser	5·3	5·2	4·3	5·3	5·0	4·1
Glu	17·7	18·9	16·2	18·6	18·8	14·1
Gly	5·0	5·5	4·5	5·3	4·8	4·0
Ala	4·9	4·8	4·0	5·1	4·9	3·8
Val	8·0	7·7	7·2	9·0	9·0	6·9
CyS	8·3	8·5	7·5	8·3	7·2	6·2
Met	Nil	Nil	Nil	Nil	Nil	Nil
Ile	1·0	1·0†	0·8	1·3	1·3*	1·0*
Leu	12·3	12·9	10·9	12·8	13·9	10·8
Tyr	10·2	9·5	9·1	10·1	9·7	8·3*
Phe	8·2	7·2	7·4	7·9	6·7	6·6*
Lys	2·9	2·8	2·3	2·6	2·8	2·3
His	5·7	5·1	4·5	5·1	5·0	4·4
Arg	3·4	3·2	2·8	3·2	3·4	2·9
Total	103·7	101·5	89·7	104·1	101·8	82·6
% of total nitrogen in the above	82	81	71	82	81	66

*Trough between peaks not down to half height.
†Trough between peaks not 0·25 inch below half height.

The conclusion was that somehow there was a loss of amino acids from the loader which must be differential because norleucine was present as an internal standard. Presumably this loss was caused by leakage, perhaps because of the high pressures sometimes required for development when the column packed down or when blockage occurred in capillaries of the loading tube. An alternative loading system was now sought which would avoid the possibility of leakage while waiting to be loaded or when loading. Systems based on a holding period in loops of P.T.F.E. tubing seemed suitable. Such a procedure of automatic loading can be provided in the Locarte Amino Acid Analyser and we were fortunate in being able to compare results using such apparatus with those from our Technicon 140 cm column. Dr W. H. Wunner of the Institute of Virology, Glasgow, kindly arranged to run our standard solution and a bean hydrolysate which we had already analysed on the 140 cm column. The results in Table 3 show that the concentrations of individual amino acids and recovery of nitrogen found can be as good on this short 24×0·9 cm diameter column loaded automatically and developed in 5 h as on the manually operated 140×0·6 cm column developed in 20 h.

Recovery of nitrogen as amino-acid nitrogen

On several occasions when we found low recovery of nitrogen from samples, loss of amino acids has been suspected. In order to check this our colleague,

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Table 3. *Proportion of nitrogen in bean hydrolysate recovered in the form of amino acids from* 140×0·6 *cm column in* 20 *h and from a* 24×0·9 *cm column in* 5 *h with automatic loading from loops of P.T.F.E.*

	Technicon 140×0·6 cm column, 20 h, manual loading		Locarte 24×0·9 cm column, 5 h, automatic loading	
Asp	6·93	7·36	7·43	7·73
Thr	2·75	3·05	2·60	2·75
Ser	4·12	4·17	3·61	3·82
Glu	10·27	9·56	10·12	10·51
Pro	0·53*	1·67*	2·84	3·29
Gly	5·36	5·57	5·30	5·45
Ala	4·65	4·58	4·03	4·23
Val	3·67	3·67	3·46	3·61
CyS	0·86	0·88	—	—
Met	0·38	0·44	0·41	0·38
Ile	2·81	2·87	2·78	2·78
Leu	5·15	4·69	4·91	5·03
Tyr	1·90	2·08	1·75	1·75
Phe	2·49	2·55	2·40	2·49
Lys	8·65	9·09	8·40	8·64
His	5·06	5·90	4·80	4·88
Arg	17·89	18·54	17·17	18·00
Recovery of N (%)	83·47	86·67	82·01	85·34

*No separation of Pro from Glu. Values calculated from ratio of absorption at 570 and 440 nm.

Mr. A. D. Hughes compared estimates of α-amino nitrogen by the Van Slyke, Dillon, MacFadyen & Hamilton (1941) ninhydrin method with those calculable from our chromatogram results. Table 4 shows good agreement in five out of the six samples, thus indicating that the chromatogram figures were reliable.

Table 4. *Comparison of estimates of free amino acid nitrogen by the Van Slyke* et al. (1941) *ninhydrin method as modified by Synge* (1951) *and by separation of individual amino acids on* 140×0·6 *cm column*

Type of hydrolysate	α-Amino nitrogen as % of total nitrogen: Van Slyke	Ion exchange
Heather I	70·4	68·6
Heather II	70·9	70·3
Heather III	68·4	66·4
Groundnut meal	73·2	73·8
Abomasal solids I	57·1	59·1
Abomasal solids II	51·5	56·1

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The determination of tryptophan in biological materials

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The determination of tryptophan in solution may be accomplished by a variety of methods. These are based upon (1) ultraviolet spectroscopy, (2) spectrofluorimetry, (3) colorimetry of the reaction with *p*-dimethyl amino benzaldehyde, and (4) chromatography followed by colorimetry of the reaction with ninhydrin.

When these methods are applied to tryptophan occurring in protein-bound form in biological materials, problems arise which have not yet been solved on a batch basis, still less by automation. In plant materials, in particular, the presence of interfering substances causes irrelevant spectroscopic absorption and prevents the application of methods (1) and (2).

Method (3) has been widely applied. In the method of Spies & Chambers (1949), the protein is solubilized by treatment with 19 N-sulphuric acid. In the method of Miller (1967), hydrolysis with barium hydroxide is employed, while the method of Lombard & de Lange (1965) uses papain to hydrolyse the protein enzymatically. All these methods depend upon the specificity of the colour reaction. In method (4) ion-exchange chromatography has been automated and provides specificity, but the requirement for hydrolysis of the protein remains and cannot yet be automated.

Hydrolysis with sodium hydroxide instead of barium hydroxide avoids mechanical and absorptive losses since the acidified hydrolysate can be applied directly to the column. The application of corrections for hydrolytic losses based upon recovery of added tryptophan is questionable, in view of the different behaviour of free and protein-bound tryptophan to hydrolysis in the presence of some reducing agents.

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Automated analysis of peptides

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The results to be given were obtained with a Technicon peptide analyser (Catravas, 1964). In our system the peptides are separated on a cation exchange resin and the