chromatogram with the butanol–pyridine–water solvent and creates no problem in this technique. The paper background of Whatman No. 1 offers very little interference in this analysis. This is of immediate interest in view of the findings of WEST[4], who has reported high concentrations of selenium in various types of paper, particularly cigarette paper.

Recovery studies have shown that 100% of selenomethionine were recovered from liver and muscle homogenates but only 60 % of the selenium added as selenomethionine to serum was recovered from the serum methionine area. The remainder of the selenium associates itself with the third ninhydrin-positive spot on the chromatogram and suggests a metabolic pathway for selenomethionine at the level of the serum.

The lower limit of detectability of this method is 0.1 μmoles of selenomethionine per g of tissue or per ml of serum. Levels of natural, free selenomethionine in rat liver, muscle and serum and in human serum apparently exist at a level lower than this because none could be detected in these sources. Consequently, this procedure is useful only for studies involving the metabolism of selenomethionine where this amino acid is added to the organism or biochemical system. A reactor with a neutron flux ten-fold or one hundred-fold greater than that used in this laboratory may increase the sensitivity of the procedure.

Special Laboratory of Nuclear Medicine and Biology, ANTHONY J. BARAK
Veterans Administration Hospital, Omaha, Nebr. (U.S.A.) STACY C. SWANBERG

1 J. L. MARTINS AND L. M. CUMMINS, *Anal. Biochem.*, 15 (1966) 530.
2 K. R. MILLAR, *J. Chromatog.*, 21 (1966) 344.
3 P. J. PETERSON AND G. W. BUTLER, *J. Chromatog.*, 8 (1962) 70.
4 P. W. WEST, *Chem. Eng. News*, 45, No. 23, (1967) 12.

Received June 6th, 1967

J. Chromatog., 31 (1967) 282–284

Rapid automatic methods for the determination of lanthionine and lysinoalanine using an amino-acid analyser

When wool is treated by a variety of setting processes, as, for example, in hot water, steam or alkalis, particular interest attaches to the fate of the cystine residues. Some of these are transformed into either lanthionine or lysinoalanine residues, thereby creating new, stable cross-linkages in the proteins of wool. The rapid and accurate assay of these two amino acids is thus of considerable benefit in the study of mechanisms of set in wool[1–4]. Of wider interest are the amounts of lysinoalanine formed during the alkali-treatment of a number of well-characterised proteins[5,6].

The work described here concerns the rapid, automatic determinations of lanthionine and lysinoalanine in protein hydrolysates, using the analytical train of the Technicon Automatic Amino-Acid Analyser[7].

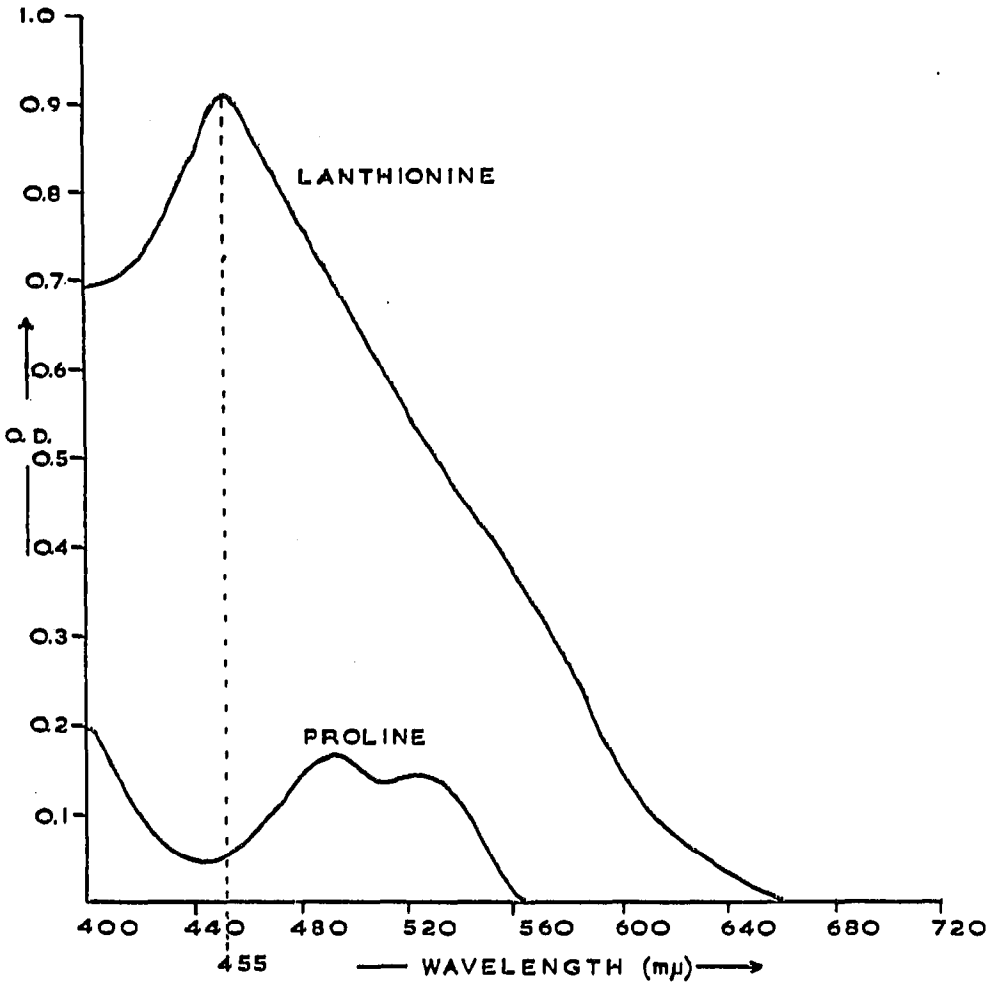


Fig. 1. Part of the absorption spectra of the coloured compounds formed when 0.265 μmoles of lanthionine and of proline react with 2.00 ml of CHINARD ninhydrin reagent for 15 min at 100°. The solutions were cooled, made up to 5.00 ml with acetic acid, and measured in 1-cm cells against reagent blanks.

Experimental

To determine lanthionine, the protein hydrolysate, between 0.5 and 2 mg, according to its lanthionine content, is applied to a cation-exchange resin column (0.6 × 50 cm, using Zeocarb 225 resin, 8% cross-linked, of nominal diameter 17μ). The column is eluted with a citrate buffer (0.05 *M*; 0.2 *M* in sodium ion; pH 3.80) at a rate of 32 ml/h. The effluent stream is mixed with a ninhydrin reagent, similar to that of CHINARD[8], prepared by mixing ninhydrin (25 g), orthophosphoric acid (400 ml) and acetic acid (1200 ml). "Acidflex" tubing must be used to pass this reagent through the proportioning pump.

After colour development in the oil bath at 93° in the usual way, the optical density of the emergent solution is measured at 450 mμ, which is close to the absorption maximum of the lanthionine derivative (Fig. 1). As shown in Fig. 2, lanthionine and proline emerge in close sequence after about 30 min of elution. The column is immediately cleansed by passing sodium hydroxide solution (0.2 *M*) for 5 min, re-equilibrated by passing citrate buffer (pH 3.80) for 30 min, and is then ready for re-

use. For calibration purposes, 0.10 μmole samples of lanthionine are eluted through the column both before and after chromatographing a succession of protein hydrolysates (Fig. 2).

A flow diagram (Fig. 3) shows the Technicon Auto Analyser set up for the the simultaneous determination of lanthionine as described and for the complete amino-acid analysis of protein hydrolysates in the conventional way. To accomodate both systems, eight tubes are employed in the manifold of the proportioning pump, together with two mixing coils, two heating coils and two cooling coils. The effluent for lanthionine assay passes through one colorimeter measuring its optical density at 450 mμ, while the effluent for complete amino-acid analysis passes through the other two colorimeters measuring at 440 mμ and 570 mμ respectively. Since lanthionine determinations are recorded only during a working day, there is little interference with the recording of the final stages of the conventional analysis, which has run throughout the previous night.

In a similar way, the resin column (0.6 × 50 cm) may be used to determine lysinoalanine. For this the column is equilibrated and eluted with a citrate buffer (0.05 *M*; 0.6 *M* in sodium ion) at pH 5.00. The remaining conditions for chromatography, colour development and recording are those recommended for the 21-h procedure used for complete amino-acid analyses[7]. Under these conditions lysinoalanine emerges just before lysine (Fig. 4; see BOHAK[6]). Norleucine, which is commonly used as an internal standard for calibration purposes, emerges with the other neutral amino

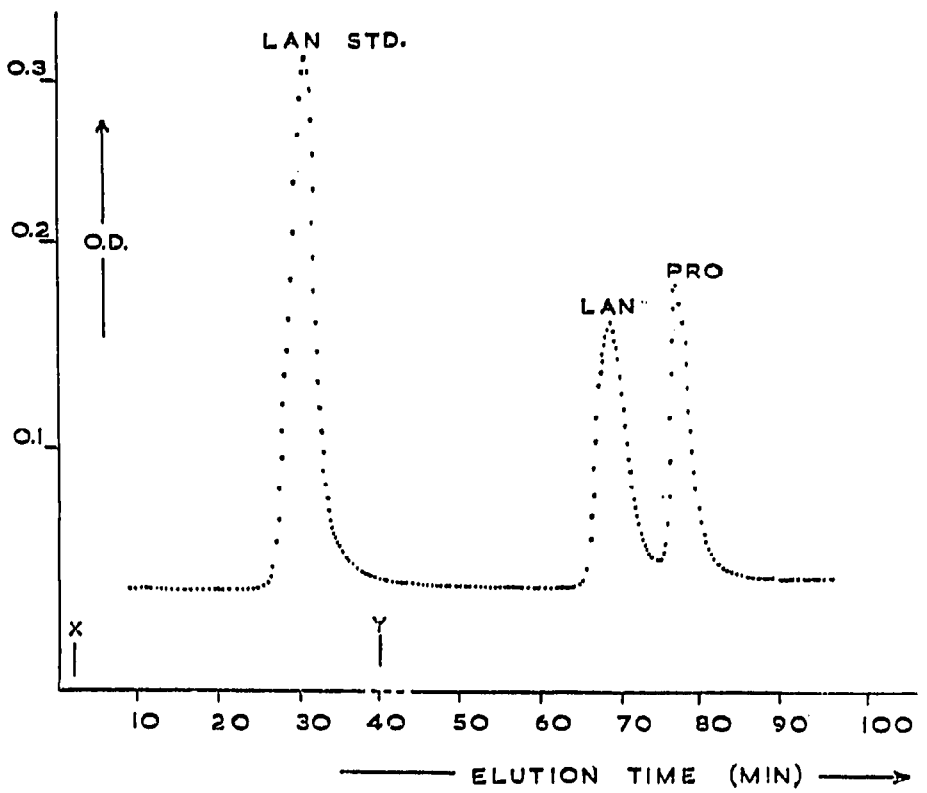
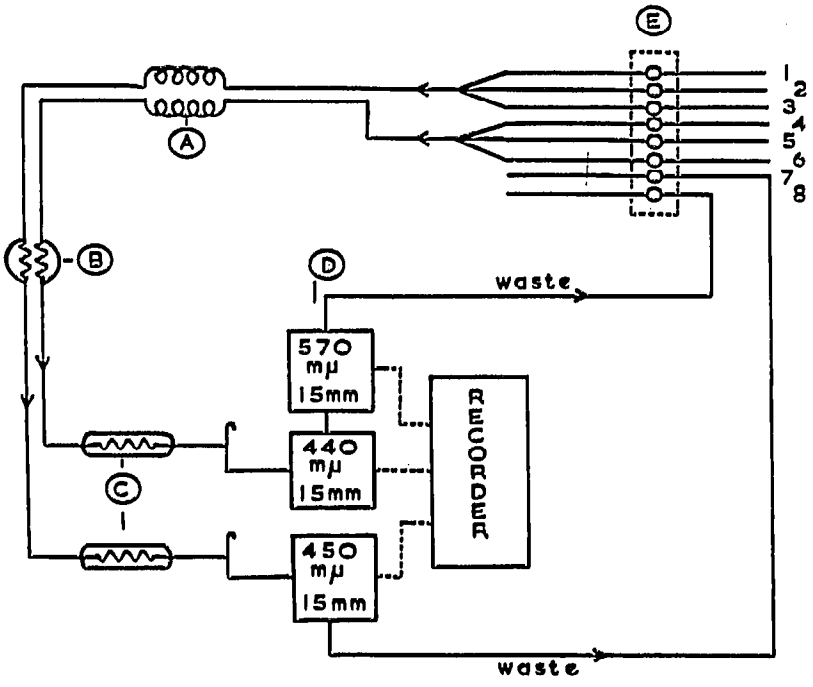


Fig. 2. Typical chromatogram obtained by applying a sample of lanthionine and a wool hydrolysate in turn to a column (0.6 × 50 cm) of Zeocarb 225 (× 8, 17 μ bead) resin and eluting with citrate buffer, pH 3.80, at 32 ml/h. 0.10 μmoles of lanthionine applied at time *X*, and 1500 μg of wool hydrolysate, containing 0.062 μmoles of lanthionine and 0.90 μmoles of proline, applied at time *Y*.



FLOW DIAGRAM
AUTOANALYSER

Fig. 3. Flow diagram of Technicon Auto Analyser arranged for both complete amino-acid analysis, and lanthionine determination. The eight tubes in the proportioning pump (E) are respectively: (1) CHINARD ninhydrin (acidflex, 0.056 in. diameter); (2) nitrogen (tygon, 0.045 in.); (3) column effluent (tygon, 0.035 in.); (4) ninhydrin–hydrindantin solution (solvaflex, 0.056 in.); (5) nitrogen (tygon, 0.045 in.); (6) effluent from long column (tygon, 0.035 in.); (7) return line (acidflex, 0.056 in.); (8) return line (solvaflex, 0.056 in.). The path followed by the mixed solutions from (1) and (3) is for lanthionine determination, and from (4) and (6) for complete amino-acid analysis. (A) are the two mixing coils, (B) two heating coils, (C) two cooling coils and (D) the three colorimeters.

acids at the beginning of the run. Until a suitable substitute, therefore, can be found, a known amount of norleucine is passed through the analytical train under the same conditions as obtain during chromatography of the protein hydrolysates. If the colour yield of norleucine is taken as 1.00, that of lysinoalanine is 1.49.

Discussion

When the Technicon Analyser is set up for complete amino-acid analysis, using a resin column, 0.6 × 130 cm, and a buffer gradient from pH 2.87 to pH 5.00, lanthionine is resolved into the *meso-* and *dl*-forms, which usually emerge between alanine and valine (*cf.* BLACKBURN AND LEE[9]). The exact elution volumes, however, are critically dependent on the precise composition of the buffers and on the flow rate, and satisfactory resolution is not always achieved. The use of citrate buffer, pH 3.80, whilst it does not resolve the diastereoisomers of lanthionine, easily separates the latter from proline; and the use of the shorter column permits faster analysis.

Including the time required for calibration, and for the necessary cleansing of the resin column between samples, five lanthionine analyses may be carried out during a working day of eight hours. In the same time, four determinations of lysinoalanine may be made. For both amino acids, over the range 0.02 to 0.20 μmoles, the accuracy is ± 3%.

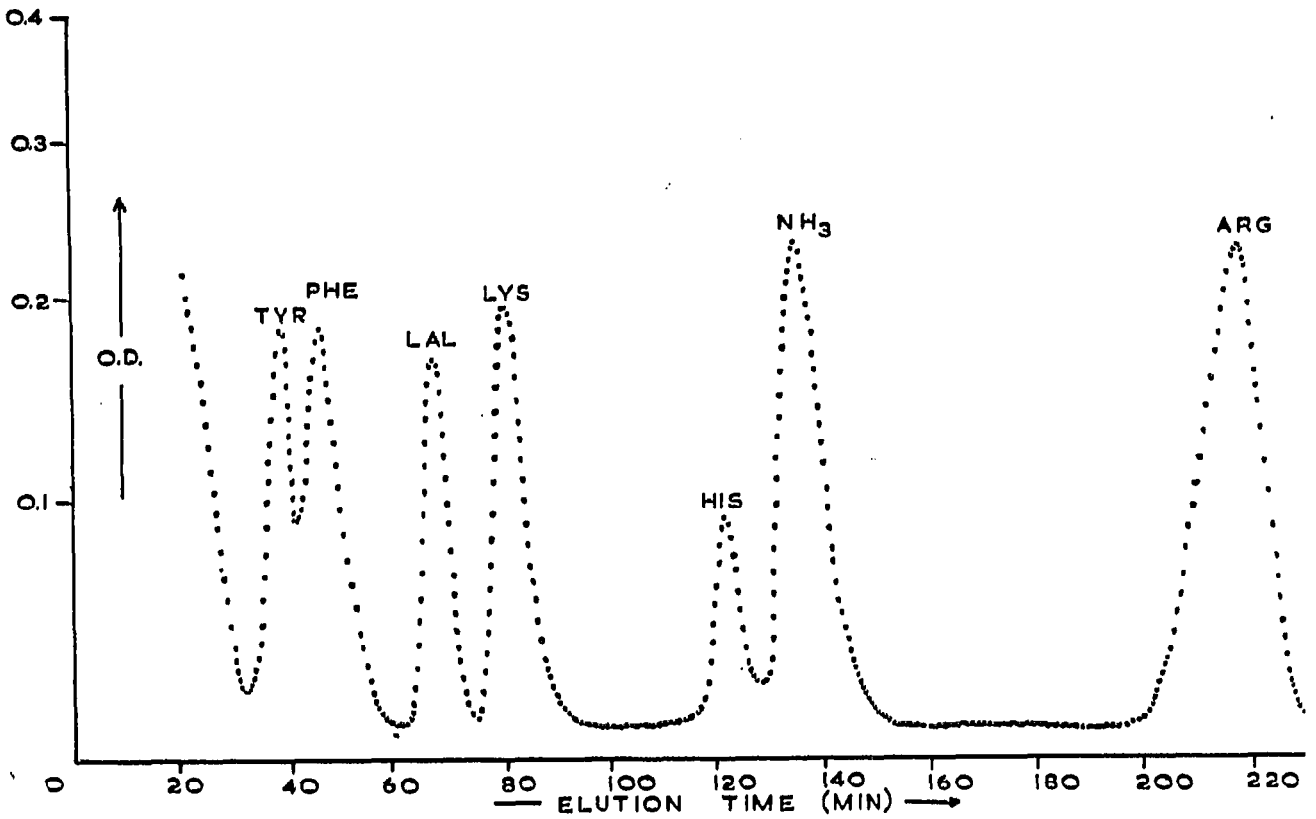


Fig. 4. Elution pattern obtained by applying a sample of a wool hydrolysate containing lysinoalanine to a column (0.6 × 50 cm) of Zeocarb 225 resin, and eluting with a citrate buffer (pH 5.00, 0.6 *M* in sodium ion) at 32 ml/h.

Acknowledgement

We wish to thank the International Wool Secretariat for a Scholarship to one of us (M.J.W.).

Department of Textile Industries, The University, Leeds 2 (Great Britain)

A. ROBSON
M. J. WILLIAMS
J. M. WOODHOUSE

1 J. B. SPEAKMAN, *Nature*, 132 (1933) 930.
2 M. EDWARDS, A. ROBSON AND C. WOOD, *3e Congr. Intern. Recherche Textile Lainière*, 2 (1965) 339
3 K. ZIEGLER, *3e Congr. Intern. Recherche Textile Lainière*, 2 (1965) 403.
4 M. C. CORFIELD, C. WOOD, A. ROBSON, M. J. WILLIAMS AND J. M. WOODHOUSE, *Biochem. J.*, 103 (1967) 15C.
5 A. PATCHORNIK AND M. SOKOLOVSKY, *J. Am. Chem. Soc.*, 86 (1964) 1860.
6 Z. BOHAK, *J. Biol. Chem.*, 239 (1964) 2878
7 *Technical Manual, Amino-Acid Analyser*, Technicon Instruments Co. Ltd., Chertsey, Surrey.
8 F. P. CHINARD, *J. Biol. Chem.*, 199 (1952) 91.
9 S. BLACKBURN AND G. R. LEE, *Analyst*, 80 (1955) 875.

Received June 2nd, 1967