Results

In Fig. 1 the petroleum ether extract from a thimble that had been preextracted with methylene chloride (chromatogram B) is compared with the extract from a thimble that had not been preextracted (chromatogram A). The arrows indicate the retention times of insecticides that could be misidentified due to the interfering materials. The response of unknown peak No. 1 is equivalent to 3 ng of dieldrin.

Thimbles also were preextracted with petroleum ether, water, methanol, ethyl ether, acetone, and acetonitrile; however, methylene chloride was the most efficient.

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Received November 5th, 1968

J. Chromatog., 39 (1969) 325–326

CHROM. 3869

Separation and estimation of collagen amino acids by programmed analysis

A rapid procedure for quantitative amino acid analysis of collagen or gelatin hydrolysates poses some problems. This protein contains 3- and 4-hydroxyprolines, usually in very different amounts, which have to be separated from each other and from aspartic acid. Another problem is the satisfactory resolution of glycine and alanine peaks, which is difficult due to unusually high quantities of glycine (one third of all amino acids). Furthermore, a good separation of only small amounts of hydroxylysine, histidine, methionine, tyrosine and occasionally methionine-sulfoxides and homoserine from neighboring peaks is required. The latter amino acid is a reaction product of methionine derived from cyanogen bromide treatment[1].

We wish to report results obtained with a rapid method for separating acidic and neutral amino acids of collagen hydrolysates. The equipment used was a "BC-200" analyzer, manufactured by Bio-Cal Instrument, Gräfelfing, Munich (Germany). This analyzer works on the basis of the approved principle described by SPACKMAN, STEIN AND MOORE[2]. It is designed for a programmed step-wise elution. A programming unit controlling five buffer systems and four different temperatures by timing devices allows the operator the choice of many separation programs that are variable over a wide range.

Our experience in separating collagen amino acids on columns of Aminex A-6 (Bio-Rad Laboratories, Richmond, Calif., U.S.A.) is summarized in Table I. A program (A) is given which takes into account all the parameters outlined in the introduction. The time required, including regeneration and equilibration of the column, is 175 min. A second program (B) is used in cases where an accurate analysis of all

TABLE I

TWO PROGRAMS FOR THE SEPARATION OF COLLAGEN AMINO ACIDS

The buffers used are citrate buffers (0.2 *M*) containing Brij 35 .Ninhydrin pumps and recorder are automatically switched off during the first and last operations (*cf.* text).

Program A			*Program B*		
pH of buffer	*Temp. (°C)*	*Time (min)*	*pH of buffer*	*Temp. (°C)*	*Time (min)*
			—	45	0
2.84	42	4	3.05	60	15
2.84	60	35	3.23	60	45
3.04	60	83	3.23	50	55
4.05	50	112	4.05	50	65
4.65	50	132	4.65	50	83
0.2 *N* NaOH	50	144	0.2 *N* NaOH	50	95
2.84	50	152	3.05	50	110
2.84	42	175	3.05	45	115

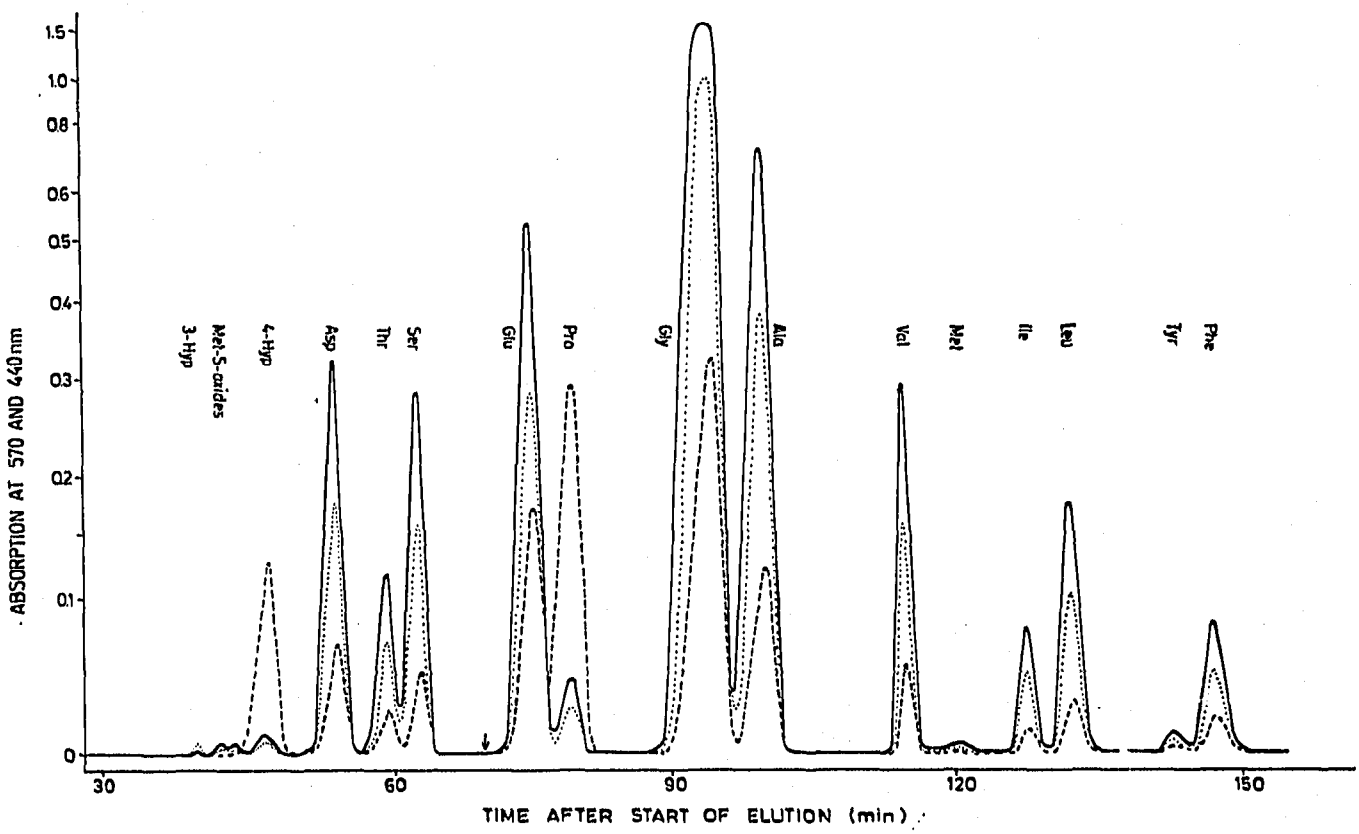

Fig. 1. Separation of collagen amino acids. Column: Aminex A-6 (59 × 0.9 cm); flow rates: 105 ml/h. for buffers and 38 ml/h. for ninhydrin. Program A (*cf.* Table I) was used for the run shown. The arrow indicates the place where homoserine is eluted. Full lines correspond to the measurement at 570 nm, dotted lines to a reduced absorption at the same wavelength. Broken lines show the absorption at 440 nm.

amino acids is not required, and preliminary experiments or routine analyses of special collagen features such as the glycine or imino acid contents may thus be performed in only 115 min. Only 3-Hyp, homoserine and the Met-S-oxides are not accounted for by program B.

Fig. 1 shows a typical elution curve of collagen amino acids obtained by using program A. 3-Hyp, the two Met-S-oxides, 4-Hyp and Asp appear as well-separated peaks if the pH of the first elution buffer is lowered to 2.84. Also at the flow rates used (105 ml/h), there are no difficulties in resolving Ser and Thr if the first buffer contains 4% (v/v) of methanol. The other three citrate buffers are free of methanol. The figure also shows that the conditions used give a good separation of Glu–Pro and Gly–Ala, in spite of the amounts present, as well as Ile–Leu and Tyr–Val.

At present the basic amino acids are eluted from a second short column (14 cm; Aminex A-5) at 50° in 85 min. The pH of the citrate buffer used is 5.28. Hyl, Lys, His, ammonia and Arg are well separated.

Approximately 0.05 micromole of an amino acid is required for an accurate analysis. This figure has to be multiplied by a factor of 2 and 4, respectively, for the determination of the imino acids Pro and Hyp. The amount of protein necessary for a total analysis is approximately 1.2 mg and may be scaled down to 1 mg if experienced workers operate the instrument. The reproducibility of the results is very satisfactory.

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Received November 6th, 1968

J. Chromatog., 39 (1969) 326–328

CHROM. 3868

The separation of maleic and fumaric acids by gel filtration on Sephadex G10

Columns of Sephadex* gels will fractionate substances according to their molecular dimensions[1]. In addition to acting as molecular sieves, these gels reversibly adsorb certain types of molecules[2]. Adsorption effects, which may be positive or negative, are particularly marked for the highly cross-linked G10 and G15 gels. Molecules with π-electron systems are adsorbed positively. Negative adsorption is shown by anions. A possible explanation for this partial ion-exclusion is that solvation makes

* Sephadex gels are cross-linked polysaccharides manufactured by Pharmacia, Uppsala, Sweden.