IN THE LABORATORY

The Quantitative Chromatography of Silk Hydrolysate

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Partition chromatography has become an important tool in the analysis of proteins. Especially as carried out on strips of filter paper according to the techniques of Consden, Gordon, and Martin (*2*), it has provided an extraordinarily rapid and sensitive way of recognizing the numerous amino acids in an hydrolysate of a protein. We have found that under proper conditions it can also provide quantitative information about the amounts of these amino acids.

As is well known, this chromatographic analysis proceeds by allowing a small volume of hydrolysate on filter paper to be leached out by a solvent saturated with water while the paper is hung up in an atmosphere saturated with vapors of this solvent and water. Under these conditions, the rates of flow of the acids present are often so different that after two or three days all of a component will be localized in a band or spot on the paper. Those acids that migrate at about the same rate in one pair of solvents can often be separated in another; or the paper can, after the first separation, be turned through 90° and the separation continued with another solvent. Since the rates of migration on an inert substrate such as paper are primarily determined by partition coefficients between the solvents used, a knowledge of these coefficients permits recognition of the acids in the bands or spots of the finished chromatogram.

Valid quantitative results can be obtained from such an analysis only if it is carried out under controlled conditions which allow comparison with a simultaneously prepared chromatogram of a known mixture of amino acids and if it involves bands well separated from one another. To obtain these, several runs, using different solvents, may be required.

There are several ways of determining the amount of amino acid in a band. It can be done by extracting the acid and making a micro-Kjeldahl determination of the extracted nitrogen. Filter paper with which we have worked has contained amounts of nitrogen comparable with that in the extracted acid, and colorimetric procedures have proved more convenient and accurate. In the techniques as finally adopted, chromatograms of the hydrolysate and of the standard mixture of acids were always prepared at the same time in the same jar. At the conclusion of the run the two were dried together without resort to heating, and color was developed by spraying the papers with ninhydrin (0.1 per cent in butanol) and heating to 90° C. for 5 minutes, using the entire paper or vertical stips cut from it to provide a guide to the positions of the bands. If entire sheets were colored, the corresponding band on the unknown and the control were cut out, their colors extracted (preferably with acetone), and the extracts made up to known volume, diluted, and compared in a colorimeter. These steps were always carried out promptly and under the same conditions for unknown and control. When the bands were sufficiently separated on the finished chromatogram so that the amino acid-containing areas of the filter paper could be identified by reference to a strip cut from its edge and colored with ninhydrin, the amino acids themselves were often leached from the paper before treatment with ninhydrin. Satisfactory results have also been obtained by applying the hydrolysate in a series of dilutions as drops and by setting up composite standards in which the known amino acid mixture was also applied at a series of dilutions. In this case, color was developed on the papers, and the amount of an amino acid in the unknown was determined by finding spots of equal intensity and area on the standard and unknown papers. This "spot-dilution" technique is especially promising.

Though knowledge of partition coefficients is often sufficient to identify the amino acids present on a chromatogram, a more direct means is frequently desirable. Electron diffraction has proved particularly useful for this purpose. If sufficient care is taken in making the preparations, excellent diffraction patterns can be obtained (*3*) with an RCA type EMU electron microscope from very few micrograms of amino acid extracted from a spot or band—and the identification this provides is unequivocal. Such diffraction observations have identified traces too small for quantitative determination and have proved useful as indices of purity of the extract from a band.

TABLE 1

THE AMINO ACIDS OF SILK

Amino acid	HCl hydrolysate of silk		Artificial mixture		Previous analyses	
	Color analysis	Spot dilution	Composition	Estimated spot dilution	(a) (*1*)	(b) (*4*)
Arginine	2.4	0.8			0.76	0.7
Glycine	39.9	42.4	43.4	39.0	43.8	40.5
Serine	12.7	11.9	1.9	3.2	13.6	1.8
Alanine	37.6	34.0	26.8	29.6	26.4	25.0
Proline	trace	trace	1.1	—	1.0	1.0
Valine	4.4	5.7			—	—
Isoleucine	trace	2.5	2.7	3.8	2.5	2.5
Phenylalanine	absent	absent	12.3	12.5	1.5	11.5
Tyrosine	5.9	8.3	11.8	14.6	13.2	11.0
Methionine	absent	absent			2.6	—
Histidine	—	—			0.1	0.1
Threonine	—	—			1.4	—
Total	102.9	105.6	100.0	102.7	106.9	94.1

This work has been carried out in the search for methods applicable to the analysis of purified viruses. To determine

the accuracy of the foregoing and other procedures tested, however, we have made many analyses of silk, chosen because of its relative simplicity and the detail with which it has been studied by classical methods of analysis. The data in Table 1 illustrate the kind of accuracy furnished by these chromatographic procedures.

The results of columns 2 and 3 are single readings of representative chromatograms. For column 2 the acids were extracted from the paper after treatment with ninhydrin and measured colorimetrically; for column 3 the procedure was that described above as the spot-dilution technique. A comparison between columns 4 and 5 indicates the kind of agreement to be expected between duplicate runs. Previous analyses are reproduced in columns 6 and 7.

There are several significant differences between our results and the earlier ones. We agree with analysis (a) and disagree with analysis (b) in finding a high serine content and little, if any, phenylalanine. According to chromatographic analysis, there is less tyrosine and more alanine than was found by earlier methods. Valine was not previously identified; on the other hand, there has been no chromatographic evidence for methionine. The solvents used in this work were not appropriate for determinations of the other two acids, histidine and threonine, that have been reported.

The methods used and results obtained in this study will be described in detail elsewhere.

References

1. COHN, E. J., and EDSALL, J. T. Composite table in *Proteins, amino acids and peptides*. New York: Reinhold, 1943. P. 358.
2. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1944, **38**, 224.
3. PICARD, R. G., and REISNER, J. H. *Rev. sci. Instr.*, 1946, **17**, 484.
4. SCHMIDT, C. L. A. Composite table in *The chemistry of the amino acids and proteins*. Baltimore: C. C. Thomas, 1944. P. 217.

Illuminator for Melting-Point Blocks

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Berl and Kullman (*2*) and Bergstrom (*1*) have described copper melting-point blocks which give more accurate results than the open beaker type of apparatus. However, the copper block is only as accurate as the amount of illumination supplied to its interior. Most investigators have used quartz rods to conduct the light to the interior. Although this gives a good source of light, the rods are expensive and subject to breakage. To overcome these disadvantages, the illuminator described below was constructed.

A piece of aluminum alloy tubing, $\frac{7}{16}$ inch in diameter, was bent to form a circle 10 inches in diameter. A piece of sheet aluminum alloy $\frac{1}{16}$ x $1\frac{1}{4}$ x 10 inches was bolted to this circle and trimmed so that the outside edge was 9 inches long and its center was $2\frac{7}{8}$ inches from the center of the block. Two cones were constructed from sheet copper. One end had a diameter of $\frac{15}{16}$ inch, and the other tapered to $\frac{5}{16}$ inch. Two brass light fixtures, each holding a 7-watt lamp, were mounted in these cones and then bolted to the aluminum ring. Wires from the lamps passed through the ring and out at the rear, where they were attached to a female plug (Fig. 1).

The illuminator was connected to the block by glass rods, $\frac{5}{16}$ inch in diameter, cut to fit the block used. The entire apparatus usually is mounted by clamping it to a ring stand. The addition of a magnifying glass or telescopic sight mounted

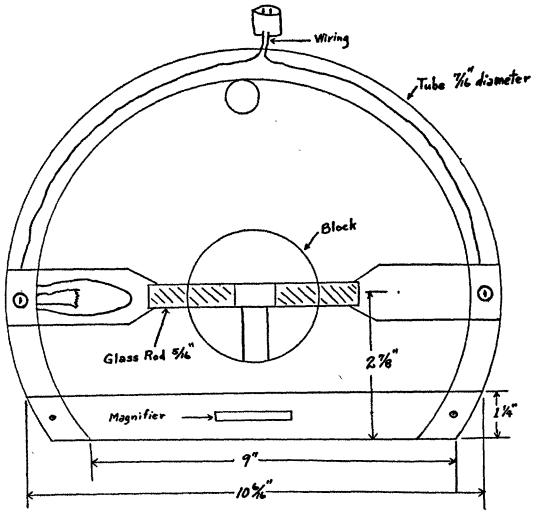

FIG. 1

in the center of the cross brace and sighted on the observation hole in the block completes the apparatus.

References

1. BERGSTROM, F. W. *Ind. eng. Chem.* (Anal. ed.), 1937, **9**, 340.
2. BERL, E., and KULLMANN, A. *Ber.*, 1927, **60**, 811.

Interference by ATP in the Estimation of Coenzyme I

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Chemical and physicochemical methods cannot be relied upon for the estimation of coenzyme I in tissue extracts; biochemical methods involving the use of enzyme systems are the most advantageous. Among these, the most widely used has been the fermentation method of von Euler and Myrbäck (*4*), where apozymase is used as the source of enzymes and the coenzyme is estimated by the volume of carbon dioxide liberated when excess sugar is fermented in the presence of inorganic phosphate and hexose diphosphate. In the method due to Warburg (*6*), partially purified enzymes, protein A and protein B, obtained from Lebedev's juice, are used, the other components of the system being coenzyme I, adenine nucleotide, Robison ester, acetaldehyde, phosphates and magnesium, manganese and ammonium ions. Jandorf, Klemperer, and Hastings (*2*) described a new method based on the catalysis by coenzyme I of the breakdown of hexose diphosphate in the presence of arsenate, a dialysed aqueous extract of cat muscle being used as the source of glycolytic enzymes. Haas (*1*) has suggested

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