SCIENTIFIC APPARATUS AND LABORATORY METHODS

MICROBIOLOGICAL DETERMINATION OF AMINO ACIDS

THE authors have found that microbiological techniques similar to those used for vitamin assays can also be used for rapid and accurate determinations of amino acids. *Lactobacillus arabinosus 17–5* appeared to be the most satisfactory of a large number of organisms tested. The following amino acids were found to be essential for the growth of this organism: glutamic acid, tryptophane, threonine, valine, leucine, isoleucine, cystine, lysine and phenylalanine.

In addition, alanine, arginine, aspartic acid, histidine, proline, serine, methionine and tyrosine increased the growth of the cultures and hence were included in the medium.

When *p*-aminobenzoic acid was added to the Snell and Wright[1] assay medium for nicotinic acid, a mixture of the above amino acids was found to adequately replace hydrolyzed casein. The growth of the bacteria on the synthetic medium was further increased by a concentrate prepared from tomato juice as described below. This concentrate appears to contain an unknown growth-stimulating factor for *Lactobacillus arabinosus*.

The active material was adsorbed from clarified tomato juice with Norite A at pH 3. Elution was effected with a pyridine, ethanol, water mixture (in the ratios 1:2:1). The eluate was evaporated to dryness and the residue hydrolyzed with 8N H_2SO_4. After removing the H_2SO_4 with $Ba(OH)_2$ the adsorption and elution was repeated.

The complete medium as used for the determination of the amino acids is based on that of Snell and Wright[1] with the casein hydrolyzate replaced by 2 milligrams of each of the above-mentioned amino acids (except glutamic and aspartic acid, which were used at a 4 mg level) and 1 mg of Norite eluate per 10 ml of completed medium. Para aminobenzoic acid was also added to the medium.

By leaving out one of the amino acids which is essential for the growth of *Lactobacillus arabinosus*, a medium for the determination of that particular amino acid is prepared. The method of conducting the tests is essentially the same as is used for the determination of nicotinic acid, titration of the amount of lactic acid formed in the test cultures being indicative of the amount of the amino acid which is present in the unknown.

The authors have found the method particularly useful for the determination of valine, leucine and isoleucine. These amino acids are sharply differentiated biologically, although their chemical structures are so similar that accurate determination by chemical means is difficult.

Pure samples of these three amino acids are more readily obtainable as the synthetic *dl* forms. In the case of valine and leucine only the naturally occurring *l* forms are active, the *d* forms being completely inactive. The *dl* forms may be used as standards, two weight units of the *dl* form being exactly equivalent in activity to one unit of the pure *l* form. Standard curves for valine determinations cover the range from 0 to 0.08 mg of *dl* valine. For leucine the range is from 0 to 0.16 mg of the *dl* form. Synthetic *dl* isoleucine can probably be used as a standard as soon as studies concerning the specificity of the bacteria for the four forms of this amino acid are complete.

TABLE I

SPECIFICITY OF *Lactobacillus arabinosus* FOR THE OPTICAL ISOMERS OF SOME AMINO ACIDS

Amino acid	Optical form	Weight per test	Titration values,* 0.1 N NaOH	
		mg	*ml*	
Valine	....	0.00	0.56	0.54
Valine	d(−)	0.02	0.52	0.54
Valine	l(+)	0.02	2.17	2.19
Valine	dl	0.04	2.19	2.19
Leucine	l(−)	0.04	2.70	2.82
Leucine	dl	0.08	2.85	2.82
Glutamic Acid	l(+)	0.02	2.78	2.82
Glutamic Acid	dl	0.04	2.78	2.82
Lysine	l(+)	0.04	2.82	2.79
Lysine	dl	0.08	2.76	2.79

* 5 ml aliquots from 10 cc culture tubes.

Table I shows the specificity of *Lactobacillus arabinosus* for the optical isomers of some of the amino acids.

A detailed report covering the application of the method to the determination of amino acids in protein hydrolyzates will be published elsewhere in the near future.

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[1] E. E. Snell and L. D. Wright, *Jour. Biol. Chem.*, 139: 675, 1941.

BOOKS RECEIVED

DAVIS, A. F. and POWERS, ED. C. *Studies in Arc Welding.* Illustrated. Pp. xxxi + 1295. James F. Lincoln Arc Welding Foundation, Cleveland, Ohio. $1.50.

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