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Microbiological Determinations of Amino Acids on the Medium of Henderson and Snell

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Henderson and Snell [1] recently described a uniform medium for the determinations of amino acids with various microorganisms *. In that paper an account of the different lactobacilli selected for the determination of fourteen amino acids was also given. In this laboratory a similar investigation has been carried out where each of eighteen amino acids was assayed with several lactic acid bacteria for which the amino acid was indispensable. Use of more than one test organism for the determination of different amino acids seems advisable depending upon the well known interrelationships between certain vitamins and amino acids and the lack of an absolute requirement.

EXPERIMENTAL AND RESULTS

Cultures and procedure. The organisms used were *Lactobacillus arabinosus* 17—5 (8014), *Lactobacillus delbrückii* LD5 (9595), *Lactobacillus casei* (7469), *Streptococcus faecalis* R (8043), *Leuconostoc mesenteroides* P-60 (8042), *Lactobacillus fermenti* 36 (9338) and *Lactobacillus citrovorum* (8081). The assay technique was mainly that described by Henderson and Snell [1]. The total volume was 6 ml. and the lactic acid was titrated with 0.05 *N* NaOH using thymol blue as indicator in the citrate containing medium and bromthymol blue in the acetate containing medium. Other small variations for carrying stab cultures and inocula are described in a recent paper (Ågren [2] 1948). In that paper it was also pointed out that according to the experience in this laboratory higher lactic acid production was obtained with *Lactobacillus citrovorum* (8081) and *Lactobacillus delbrückii* LD5 when they were grown in a medium containing 0.5 times the concentration of all ingredients of the Henderson and Snell medium with the exception of Salt C which was kept at the original concentration. Accordingly, these two microorganisms were cultivated on this weaker medium. *Lactobacillus casei* was usely grown in the original

* I wish to express my appreciation to these authors for obtaining the composition of their basal medium prior to its publication.

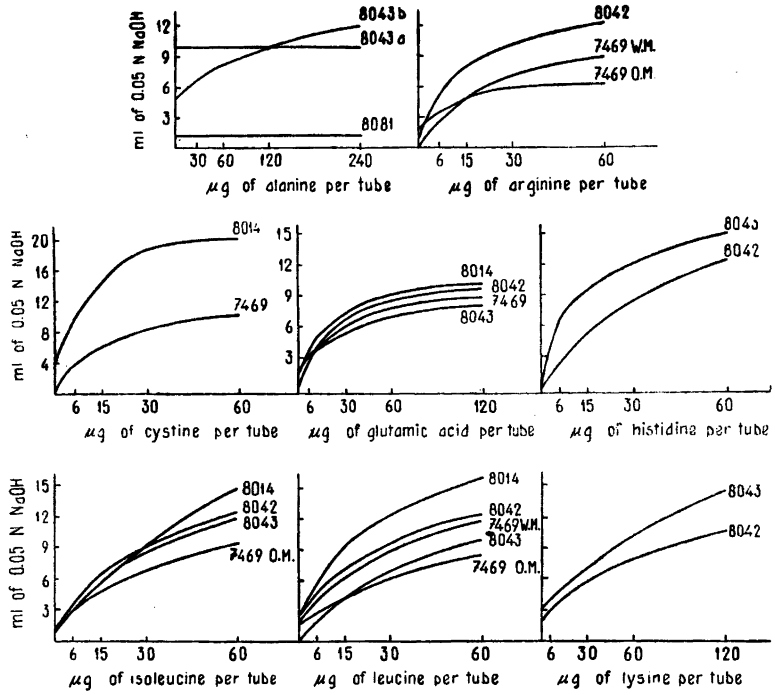

Figure 1. Standard curves on the medium of Henderson and Snell. The American Type Culture Collection number is used for each microorganism.

medium (O. M.) but for some amino acids more steep standard curves were obtained when the weaker medium was used (W. M.). The amino acids used were from Merck. DL-forms of alanine, aspartic acid, isoleucine, methionine, phenylalanine, serine, threonine and valine were employed. The natural isomers of the others were used. For some amino acids (alanine and glycine) the medium of Dunn *et al.*[3] was prefered.

Standard curves. Results obtained with the different amino acids tested on the medium of Henderson and Snell are given in Fig. 1 and 2. When *Streptococcus faecalis* R was grown on the original medium of Henderson and Snell, curve 8043 a, alanine was a dispensable amino acid for the microorganism. When the citrate buffer was exchanged for acetate, curve 8043 b, better growth curves were obtained. The high level of the curve may depend on the presence of the pyridoxine derivate in the medium (Lyman *et al.*[4] 1947). Dunn *et al.*[3] also reported that alanine was an essential amino acid for *Streptococcus faecalis* R but they also obtained a comparatively high blank value on the medium which contained acetate but no citrate. For corroboration of this result see Fig. 3. It appears that *Streptococcus faecalis* R has the enzymatic

mechanism for synthesezing alanine provided that the proper substrates are present. The poor growth curve with *Lactobacillus citrovorum* in the medium of Henderson and Snell was previously reported (*cf.* Ågren [2] 1948).

Comparing the standard curves obtained with different lactobacilli when arginine was obmitted from the medium *Leuconostoc mesenteroides* gave the steepest curves. *Lactobacillus casei* seemed to give a better curve when the weaker medium of Henderson and Snell was used. Of the seven microorganisms tested in the present investigation only *Leuconostoc mesenteroides* could be used for aspartic acid determinations (*cf.* Dunn *et al.*[3], Henderson and Snell [1]). The cystine standard curves obtained with *Lactobacillus arabinosus* were always much steeper than those obtained with *Lactobacillus casei.* In some experiments when *Lactobacillus arabinosus* was used for cystine determinations it was observed that more consistent results were obtained after 48 hours of incubation than after 72 hours.

The glutamic acid curves in Fig. 1 were all obtained in experiments were the microorganisms were cultivated on the weaker medium of Henderson and Snell adjusted to pH 7.0. In this case no lag period was encountered. Using the medium in the same concentration as given by Henderson and Snell the induction period observed by this authors was also noticed in this laboratory. The medium seemed not to be suitable for assays of glycine with *Leuconostoc mesenteroides* or *Lactobacillus citrovorum.* The same lactic acid production was obtained whether glycine was present in the medium or not. Exchanging citrate for acetate made no difference. Better assay curves were obtained on the medium of Dunn *et al.*[3] (Fig. 3). Subsequently acceptable curves were also obtained on the medium of Henderson and Snell when recrystallized alanine was used (*cf.* Henderson and Snell [1], Shankman [5]). With regard to histidine it may be mentioned that *Streptococcus faecalis* R was used by Henderson and Snell in their analysis of this amino acid. The same authors also reported severs drifts in assay values when *Lactobacillus arabinosus* was employed for isoleucine determinations. The levels of the leucine curves obtained with *Lactobacillus casei* were always higher when the diluted medium of Henderson and Snell was used (Fig. 1).

For several reasons (*cf.* Lyman *et al.*[4], Dunn *et al.*[3], Stokes *et al.*[6]) only *Streptococcus faecalis* R and *Leuconostoc mesenteroides* could be used for lysine determinations. Methionine assays carried out with several microorganisms on the diluted medium of Henderson and Snell always gave much lower standard curves than assays performed with the original medium. Only the latter curves are shown in Fig. 2. *Lactobacillus arabinosus* has been used by several investigators for determination of phenylalanine but this microorganism and *Streptococcus faecalis* (9790) may be able to synthesize phenylalanine (cf.

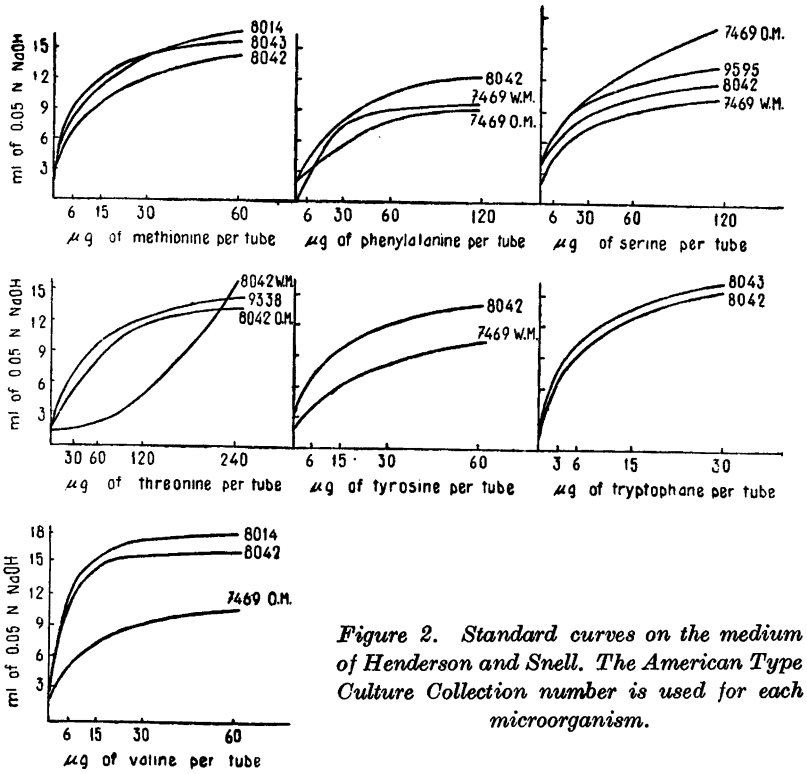

Figure 2. Standard curves on the medium of Henderson and Snell. The American Type Culture Collection number is used for each microorganism.

Stokes *et al.*[6], Lyman *et al.*[4]). A steeper phenylalanine curve was also obtained with *Lactobacillus casei* when the microorganism was cultivated on the diluted medium than on the original (*cf.* Fig. 2). Of the seven microorganisms employed in the present investigation only *Leuconostoc mesenteroides* seemed to be suitable for proline determinations (Snell [7]) and this lactobacillus was also used by Henderson and Snell. Dunn *et al.*[3] showed that on their medium this microorganism could not use hydroxyproline instead of proline.

Serine standard curves were carried out with *Lactobacillus casei* and *Leuconostoc mesenteroides*. The composition of the medium influences the types of curves obtained. Contrary to the previous experience with *Lactobacillus casei* a steeper curve was obtained when the microorganism was cultivated on the original medium of Henderson and Snell. Meinke and Holland [8] recently demonstrated that 2 mg of DL-threonine per 10 ml of the basal medium had an antagonistic effect on the utilization of serine by several lactic acid bacteria. The inhibitory effect was stronger when *Lactobacillus casei* or *Lactobacillus delbrückii* were used as test organisms while the effect

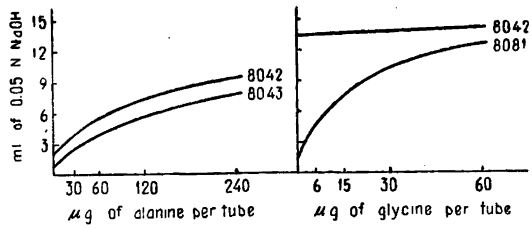

Figure 3. Standard curves on the medium of Dunn, Shankman, Camien and Block. The American Type Culture Collection number is used to denote each microorganism.

was less disturbing in assays with *Leuconostoc mesenteroides* or *Streptococcus faecalis*. In the medium of Henderson and Snell the inhibitory effect was also observed. When threonine was excluded from the medium much steeper standard curves were obtained with *Lactobacillus casei* and *Lactobacillus delbrückii*. Only the latter types of curves are shown Fig. 2.

The two different types of threonine curves obtained with *Leuconostoc mesenteroides* when it was grown in the two concentrations of the Henderson and Snell medium is of interest. Meinke and Holland [8] found that serine had an antagonistic effect on the utilization of threonine and that this amino acid inhibited the availability of serine for growth. Now, the threonine standard curves obtained by these authors with *Leuconostoc mesenteroides* shows the same type of induction period as the threonine curves obtained by us in the diluted medium of Henderson and Snell while this lag disappears when the original medium is used. Thus it would seem possible to eliminate this lag by using a suitable medium. The experience obtained in this laboratory is also that the serine-threonine antagonism is more pronounced on the medium of Meinke and Holland than on the medium of Henderson and Snell.

As mentioned before the exchange of citrate for acetate in the medium of Henderson and Snell made alanine an essential amino acid for *Streptococcus faecalis*. As the medium of Dunn *et al.*[3] only contained acetate it was considered of interest to carry out some alanine assays on that medium. Typical results are given in Fig. 3. The curves were of the same flat type as those obtained on the medium of Henderson and Snell but the blank values were lower. Attempts were also made to obtain suitable glycine standard curves on the medium of Dunn *et al.*[3]. *Leuconostoc mesenteroides* and *Lactobacillus citrovorum* were used as test organisms. The Merck DL-alanine preparation used in the medium was not recrystallized and the two glycine curves shown in Fig. 3 were carried out simultaneously on the same batch of medium. The curve obtained with *Leuconostoc mesenteroides* was of the same horizontal type as that obtained on the medium of Henderson and Snell. *Lactobacillus*

citrovorum on the latter medium also gave a simular curve. The reason for the different result obtained with *Lactobacillus citrovorum* on the medium of Dunn *et al.*[3] must depend on differences in the composition of the two media and not on a possible glycine contamination in the alanine preparate. Our results with the two microorganisms on the medium of Dunn *et al.*[3] are in agreement with those reported by these authors. One consequense is obviously that commercial alanine preparations may be used without crystallization for glycine assays if these are carried out on the medium of Dunn *et al.*[3] with *Lactobacillus citrovorum.*

SUMMARY

Using seven lactic acid bacteria and the uniform medium of Henderson and Snell several standard curves have been carried out for each of the eighteen amino acids which can be determined by microbiological methods. This was made to complete the investigation of Henderson and Snell which comprised fourteen amino acids and to obtain a conception of alternatively usable microorganisms. With one exception the reported results of Henderson and Snell included the use of one microorganism for each amino acid. Employment of more than one test organism seems advisable depending on the well known interrelationship between certain vitamins and amino acids and the lack of an absolute requirement. The implication of some results are discussed.

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REFERENCES

1. Henderson, L. M., and Snell, E. E. *J. Biol. Chem.* **172** (1948) 15.
2. Ågren, G. *Acta Physiol. Scand.* (1948) In press.
3. Dunn, M. S., Shankman, S., Camien, M. N., and Block, H. *J. Biol. Chem.* **168** (1947) 1.
4. Lyman, C. M., Mosely, O., Wood, S., Butler, B., and Hale, F. *J. Biol. Chem.* **167** (1947) 177.
5. Shankman, S. *J. Biol. Chem.* **173** (1948) 809.
6. Stokes, J. L., and Gunnes, M. *Science* **101** (1945) 43.
7. Snell, E. E. *Ann. New York Acad. Sc.* **47** (1946) 161.
8. Meinke, W. W., and Holland, B. R. *J. Biol. Chem.* **173** (1948) 535.

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