The Toxicity of Small Concentrations of Cystine to Acid-producing Bacteria

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SUMMARY: None of the various methods proposed for the microbiological assay of cystine is entirely satisfactory. *Leuconostoc mesenteroides* P-60 appeared to be the most suitable micro-organism, but assays were nevertheless variable and, occasionally, completely unreliable. A toxic effect characterized all assays to some extent, but no satisfactory explanation of its occurrence can be advanced.

As yet no entirely satisfactory microbiological method for the assay of cystine has been reported. Assays are described, using *Lactobacillus arabinosus*, by Shankman, Dunn & Rubin (1943) and by Barton-Wright (1946), but the absolute requirement of cystine by *L. arabinosus* has not been confirmed by Riesen, Spengler, Robblee, Hankes & Elvehjem (1947) or by work in this laboratory (unpublished data). With oxidized-peptone medium, Lyman, Moseley, Wood & Hale (1947) found that, though smooth and regular standard curves could be obtained for methionine, tyrosine and tryptophan on media in which most of the amino-acids required were supplied by oxidized-peptone, standard curves for cystine using the same organism (*L. casei* ϵ , *L. helveticus*) proved to be irregular and inconsistent. Similar conclusions about the non-reproducibility of standard curves for cystine with this organism were reached by Shankman *et al.* (1943). Dunn, Shankman, Camien, Frankl & Rockland (1944) stated that cystine could be determined by *Leuconostoc mesenteroides* P-60, and Riesen *et al.* (1947) concluded that this organism is the most suitable for the determination of this amino-acid.

Assays of cystine carried out in this laboratory with *Leuconostoc mesenteroides* P-60 on oxidized-peptone medium or a known mixture of amino-acids yielded standard curves which varied in range of titre from assay to assay. No such effects were observed when methionine or tyrosine were assayed using the same organism and similar media, and the assay range, as shown by higher titres, was greater in either case than in assays of cystine under the same conditions. With regard to media for cystine assays, oxidized-peptone is to be preferred since blanks are usually lower than those obtained on chemically defined media (amino-acid mixtures). Riesen *et al.* (1947), also, have demonstrated the superiority of oxidized-peptone medium for the assay of cystine, using several organisms for which cystine is said to be essential.

There is a further complication due to an apparent toxic effect, characterized by a slight break in the assay curve but also shown by a clumping of the bacteria. Fig. 1 shows a standard curve for cystine with rather better than average titres, compared with a normal standard curve for methionine. The same oxidized-peptone medium was used in each case, with the appropriate omissions of methionine and cystine respectively. The clumping of the bacteria results in

the formation of a solid cone the base of which clings to the bottom of the test-tube, whilst the mass of bacteria swirl round and round the tube when the latter is shaken with a rotatory movement. With a healthy culture of cells, this same movement would disperse the cells evenly throughout the tube. Several possibilities which might explain the phenomenon were investigated as follows.

Fig. 1. Dose-response curves of *Leuconostoc mesenteroides* P-60, produced by additions of DL-methionine and DL-cystine, respectively, to the same (oxidized-peptone) medium.

Cysteic acid. The presence of this substance might account for toxicity on oxidized-peptone medium but would not explain the effect in the chemically defined medium. Further, cysteic acid is present during assays of methionine and tyrosine using oxidized-peptone medium without harmful effects to the bacteria.

D-Cystine. The toxicity might result from the D-isomer of the amino-acid, since DL-cystine was used in preparing the standard solution. Comparable assays carried out under exactly the same conditions and at the same time showed little difference in the form of standard curve or in the assay range, whether the natural isomer (L) or the racemic mixture (DL) as in the synthetic product was used. Since only the L-isomer is active, standard solutions of DL-cystine were used in twice the concentration of those of L-cystine for comparable assays. In both cases the full toxic effect was observed.

Deficiency due to lack of SH-groups. Though similar cases of bacterial clumping with *Leuconostoc mesenteroides* on nutritionally complete media have been observed in this laboratory (unpublished data), it was considered desirable to test whether a deficiency of SH-groups might be operative. The argument can legitimately be raised since, in the assay of methionine, a concentration of 100 μ g. of L-cystine/10 ml. of medium is present, whereas the highest concentration of the standard cystine corresponds to a concentration of only half this amount. The addition of thiolacetic acid in amount equivalent to that of L-cystine (0.1 g./l.) contained in the medium for methionine assay, however, produced only a very slight lowering of the titres. No diminution in toxicity was observed.

Finally the possibility cannot be entirely excluded that bacterial clumping itself might be the cause rather than the result of the apparent toxicity.

Bacterial clumping was also observed in assay tubes of media containing protein-hydrolysates as well as in the standard tubes. It takes place after incubation at 37° for 16 hr. It would appear, *prima facie*, that L- and DL-cystine are toxic to *Leuconostoc mesenteroides* P-60 (and possibly to other lactic acid-producing bacilli) when added in small amounts (e.g. 0–50 μ g./10ml. for L-cystine) to media otherwise nutritionally adequate for the organism. This toxicity is independent of the need of cystine for growth and probably accounts for the variability in assay range in the microbiological assay of cystine. Caution in accepting figures resulting from one or two assays only would appear to be necessary.

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