Note on the fluorimetric method for phenylalanine in serum

The McCaman and Robins procedure[1] for the fluorimetric determination of phenylalanine in serum was modified by Wong *et al.*[2]. In order to stabilize the pH they proposed to use a more concentrated succinate buffer and to add bovine serum albumin to the standard solutions of phenylalanine.

In our hands the modified procedure resulted in about 25% lower values, not due, however, to differences in pH. Both the concentrated and the diluted succinate buffer resulted in the same pH of the reaction mixture before the addition of the copper reagent. When albumin was added to the standard solution of phenylalanine, the pH of the reaction mixture proved to be the same as was the case without added albumin.

On further investigation we found the fluorescence to be strongly dependent on the trichloroacetic acid (TCA) concentration. This effect of varying TCA concentration upon the relative fluorescence is shown in Fig. 1.

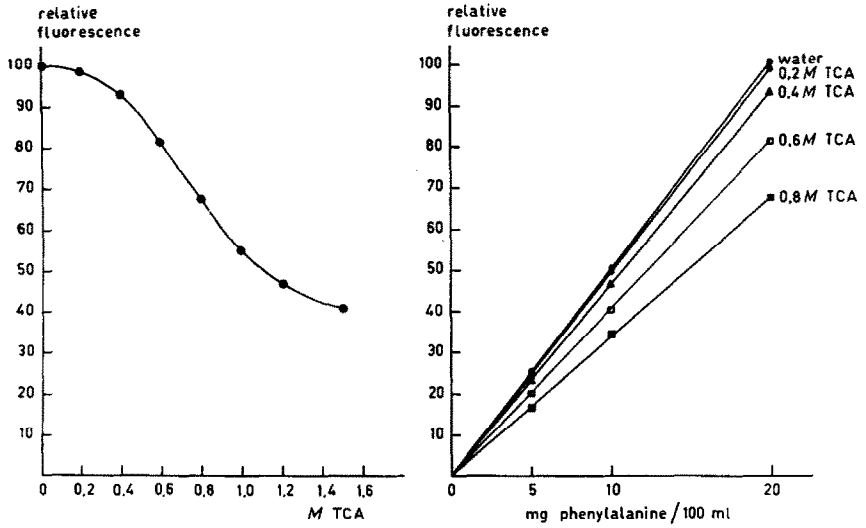

Fig. 1. The influence of varying TCA concentration on the relative fluorescence. Phenylalanine dissolved in water. Concentration 20 mg/100 ml. One vol. of the phenylalanine standard was mixed with 1 vol. of TCA of the given concentration. Succinate buffer concentration 0.6 *M*. The pH of the reaction mixture was 5.75 in all cases.

Fig. 2. Relationship between phenylalanine concentration and fluorescence in the presence of varying TCA concentration.

The curves in Fig. 2 demonstrate a linear relationship between the fluorescence and the phenylalanine concentration in all cases. The graphs all pass through the origin.

As the TCA concentration in a deproteinized serum sample depends on the protein content of the serum, it is apparent that the relative fluorescence will vary with the protein content of the sample. Fig. 3 demonstrates the effect of varying protein concentrations of the sample upon the relative fluorescence.

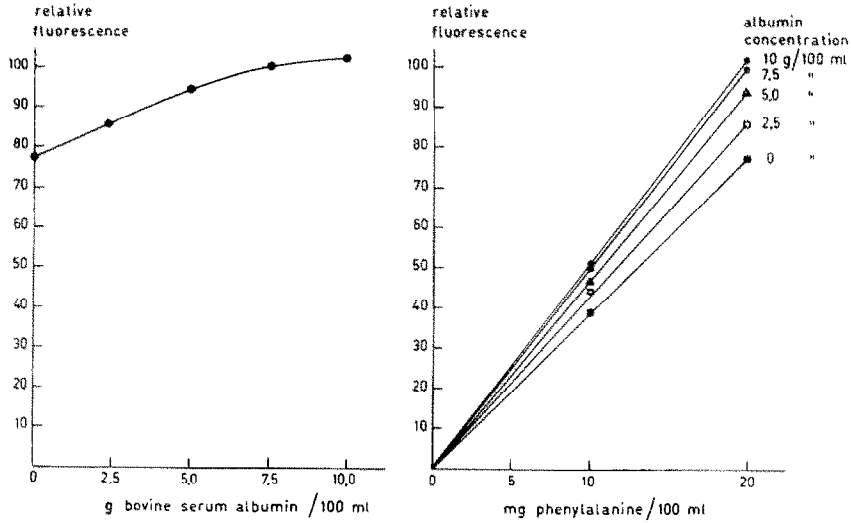

Fig. 3. The effect of varying bovine serum albumin concentration in the sample on the relative fluorescence. Phenylalanine dissolved in bovine serum albumin solution. Phenylalanine concentration 20 mg/100 ml. TCA concentration 0.6 *M*. The pH of the reaction mixture 5.75 in all cases.

Fig. 4. Relationship between phenylalanine concentration and fluorescence in the presence of varying bovine serum albumin concentration in the sample.

The curves of Fig. 4, all passing through the origin, again demonstrate a linear relationship between fluorescence and phenylalanine concentration.

The increasing fluorescence in relation to increasing albumin concentration was not found to be caused by some fluorescent or fluorescence-enhancing material in the albumin solution. This was demonstrated firstly by the fluorescence of the blank which had the same value as when the albumin solution was replaced by water and, secondly, by measuring the relative fluorescence of ultrafiltrates of standard solutions (with added albumin) in the method. The fluorescence had the same value as given by phenylalanine solutions in water without added albumin. The ultrafiltrates were prepared by pressing the solutions through a collodium membrane.

Ambrose *et al.*[3] state that impurities in the trichloroacetic acid interfere by quenching the fluorescence. They advise to filter the TCA solution through a Millipore filter to remove the impurities and, in addition, they diluted the deproteinized sample with water. We found, however, that even with a filtered TCA solution the fluorescence was dependent on the TCA concentration. Though the fluorescence increased when a filtered TCA solution was used, it is probable that this is caused by some material originating from the filter, for the same effect was obtained when the TCA solution was shaken with some filters.

When ethyl alcohol was used as deproteinizing agent, the relative fluorescence was found not to depend on the protein content of the samples (Fig. 5). Again the relationship between the fluorescence and the phenylalanine concentration is linear (Fig. 6).

Ethyl alcohol as deproteinizing agent in the method seems to make it more imperative to use a blank in which leucylalanine is omitted from the reaction mixture, than is the case when using TCA.

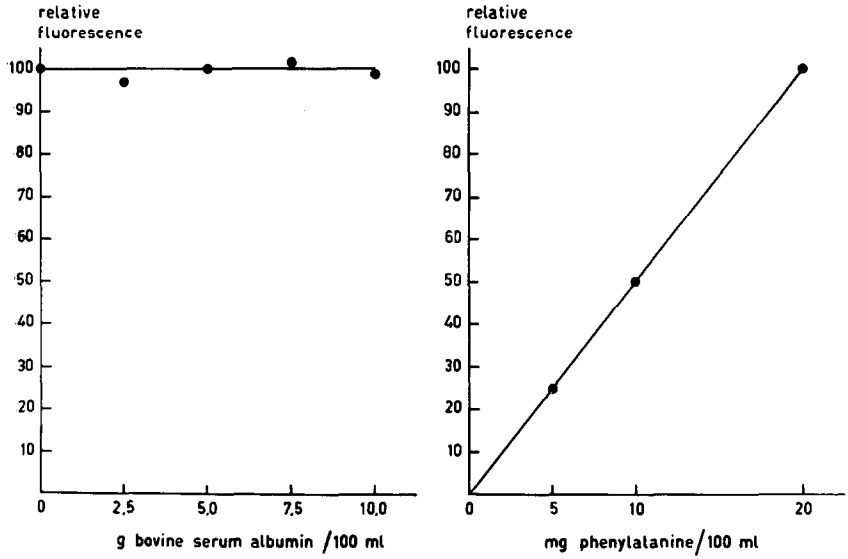

Fig. 5. The influence of varying bovine serum albumin concentration in the sample on the relative fluorescence. Phenylalanine concentration 20 mg/100 ml. One vol. of sample was mixed with 3 vol. of absolute ethyl alcohol. Mixture allowed to stand 10 min or more before centrifuging.

Fig. 6. Relationship between phenylalanine concentration and fluorescence in the presence of bovine serum albumin (0–10 g/l) in the sample when ethyl alcohol is the deproteinizing agent.

To summarize, the following conclusions can be drawn:

1. In the fluorimetric method the use of protein-free phenylalanine standards leads to erroneous results if TCA is used as deproteinizing agent. If standard solutions with added albumin are used, small errors are to be expected when the protein concentration of the sample differs from the protein concentration of the standard solutions.

2. To avoid influences of the protein content of the samples on the results, deproteinization with ethyl alcohol is preferable. In this case no albumin needs to be added to the phenylalanine standard solutions.

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