THE DETERMINATION OF TYROSINE IN PROTEINS.

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(Received for publication, September 24, 1918.)

In the determination of the amino-acids in proteins, the tyrosine is usually estimated by hydrolyzing a separate 50 gm. portion of the protein in addition to the larger quantity used for the esterification. The hydrolysis may be accomplished by means of either sulfuric or hydrochloric acid. In most instances sulfuric acid has been employed. After the hydrolysis the sulfuric acid is removed quantitatively by means of barium hydroxide. This results in a large precipitate of barium sulfate which must be washed repeatedly to remove all of the tyrosine. The tyrosine, together with the other amino-acids, is thus left in a large volume of water which must be concentrated before the tyrosine will crystallize out.

When hydrochloric acid is used the hydrolysate is filtered to remove humin and the filtrate is decolorized with carbon. It is then concentrated under diminished pressure to a sirup in order to remove as much hydrochloric acid as possible. The residue is diluted to 1 liter with water. The chlorine content is determined in an aliquot part and hydrochloric acid is neutralized by adding the calculated quantity of potassium hydroxide. The final volume of solution thus obtained is less than when sulfuric acid is used and there is no precipitate to adsorb the tyrosine. This method is therefore the more convenient and less laborious.

In a recent hydrolysis of kafirin, the alcohol-soluble protein of kafir, Andropogon sorghum (1), determinations of tyrosine were made by both methods and the quantities found were 2.48 and 2.45 per cent, respectively. In both determinations the solutions were gradually concentrated with the removal of successive crops of tyrosine until only traces of tyrosine crystallized out, together
with considerable quantities of other amino-acids. Nevertheless, the mother liquors still gave tests for tyrosine with Millon's reagent showing that all of the tyrosine had not been isolated.

A third determination was therefore made using the colorimetric method of Folin and Denis (2) after hydrolyzing 1 gm. of kafirin by boiling for 12 hours with 25 cc. of 20 per cent hydrochloric acid. This determination indicated the presence of 5.49 per cent of tyrosine in kafirin.

When tyrosine is to be isolated from a protein it is customary to boil the protein with the mineral acid for from 18 to 30 hours or even longer. It is questionable whether it is advisable to boil for so long a period. Gortner (3) has shown that under certain conditions tyrosine is slowly decomposed when a protein is boiled with hydrochloric acid. In order to ascertain whether more tyrosine can be isolated by decreasing the duration of the hydrolysis, a 50 gm. sample of kafirin was hydrolyzed for 12 hours with 20 per cent hydrochloric acid. In this experiment 3.91 per cent of tyrosine was isolated. This is 1.43 per cent more than was obtained when kafirin was hydrolyzed for 48 hours. It therefore seems unnecessary and even detrimental to continue the hydrolysis for more than 12 hours. It is very unlikely that all of the tyrosine was isolated in this experiment since it is practically impossible to crystallize out all of the tyrosine from the hydrolysates of most proteins. It is therefore probable that the percentage of tyrosine indicated by the colorimetric method of Folin and Denis represents more accurately the quantity of tyrosine present in the protein than does the percentage obtained by direct isolation.

Objections have been raised by Abderhalden and Fuchs (4) and Abderhalden (5) to the colorimetric method of Folin and Denis on the ground that tryptophane, oxytryptophane, and \( L \)-oxypoline, if present, will cause high results. These authors, however, report only qualitative tests with the above amino-acids and do not consider the effect of hydrolysis. It is true that tryptophane gives a blue color with the reagent of Folin and Denis, but the intensity of the color is much less than that given by an equivalent weight of tyrosine. It is well known that tryptophane is decomposed by acid hydrolysis. To ascertain whether this decomposition is complete and that the decomposition products do not give a color with the reagent of Folin and
Denis, the following experiment was made: 5 per cent of tryptophane was added to 0.5 gm. of kafirin and the mixture was boiled with 20 per cent hydrochloric acid for 12 hours. The tyrosine in the hydrolysate was estimated by the method of Folin and Denis and was found to be 4.36 per cent. A hydrolysis of kafirin performed under similar conditions without the addition of tryptophane gave 4.84 per cent of tyrosine. Hence the tryptophane was completely decomposed and its decomposition products gave no blue color with the reagent of Folin and Denis. It is also to be expected that oxytryptophane, if present, would be decomposed by acid hydrolysis, since the presence of the hydroxyl group would probably render it less stable than tryptophane. A sample of gelatin to which tryptophane had been added was also hydrolyzed. The blue color obtained with the reagent of Folin and Denis was of the same intensity as that obtained by a control hydrolysis where no tryptophane was added. The faint blue color obtained from the hydrolysate of the gelatin was probably due to tyrosine since the gelatin gave a distinct test for tyrosine with Millon's reagent.

Abderhalden (5) states that l-oxyproline gives a blue color with the reagent of Folin and Denis. On the other hand, Folin and Denis (2) obtained only a faint blue color from the hydrolysate of gelatin which contains 3 to 6 (6) per cent of oxyproline. This color was probably due to tyrosine. We tested a number of high grade samples of gelatin and did not find one that did not respond to the test for tyrosine with Millon's reagent. Even gelatin prepared from carefully cleaned cartilaginous rings of ox trachea gave a decided test for tyrosine.

Tyrosine and cystine are the least soluble in water of all the amino-acids obtained from the hydrolysate of a protein. Most of the cystine present in proteins is decomposed by prolonged acid hydrolysis and does not interfere with the isolation of tyrosine from most proteins. On the other hand, the presence of considerable quantities of other amino-acids in a hydrolysate may render the tyrosine readily soluble. We encountered a case in which considerable tyrosine was separated from a hydrolysate. After this tyrosine had been removed a large crop of almost pure leucine separated. On concentrating the mother liquor considerably more tyrosine mixed with a little leucine was obtained. In an-
other instance we had a similar experience with a fraction of a hydrolysate from which the leucine and some valine had been removed and which on further concentration still yielded some pure tyrosine.

CONCLUSIONS.

The method of Folin and Denis for the determination of tyrosine has been investigated. It has been found that tryptophane is completely decomposed during the hydrolysis of proteins with hydrochloric acid and that the decomposition products do not interfere with the determination of tyrosine by the method of Folin and Denis. Since tyrosine is decomposed to some extent during hydrolysis there seems to be no advantage in hydrolyzing more than 12 hours. It has been shown that oxyproline does not interfere with the determination of tyrosine by the method of Folin and Denis. Gelatin which is said to contain up to 6 per cent of oxyproline gave but little color with the reagent of Folin and Denis after hydrolysis. This color was probably due to tyrosine in the gelatin since a test for tyrosine was obtained by Millon's reagent.

BIBLIOGRAPHY.

2. Folin, O., and Denis, W., J. Biol. Chem., 1912, xii, 245.
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J. Biol. Chem. 1918, 36:319-322.

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