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XXII. HERZIG AND MEYER'S REACTION APPLIED TO PROTEINS AND AMINO-ACIDS.

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This reaction [Herzig and Meyer, 1894] has been known for a considerable time as a means of detecting the presence of, and estimating, methyl groups attached to nitrogen in organic compounds. It consists in heating the substance to a high temperature in the presence of excess of hydriodic acid, so that the methyl groups leave the nitrogen to form methyl iodide, and the hydrogen of the hydriodic acid takes the place of the methyl group. The methyl iodide is then carried over into alcoholic silver nitrate, where silver iodide is formed. The precipitate of silver iodide can be weighed and from its amount the percentage of methyl attached to nitrogen can be calculated.

Certain precautions have to be taken in the estimation of methylated nitrogen in this way. Thus substances containing methoxy groups give a silver iodide precipitate, according to the well known Zeisel reaction. The precipitate from methoxy groups, however, forms when the reaction mixture is heated to 130°, whereas that from methylamino groups requires a temperature of 230°. In connexion with methoxy derivatives it must also be remembered that glycerol and fats (on account of their glycerol content) give rise to isopropyl iodide and to a silver iodide precipitate. Trier has published a criticism [Trier, 1913] of the method stating that amino-ethyl alcohol gives a precipitate under these conditions.

In the course of ordinary estimations by this method, it was found that commercial gelatin gave a positive result. At the end of the experiment the precipitate formed in the flask containing silver nitrate was black. This was due to the sulphur in the protein, for on boiling with dilute nitric acid, the black colour disappeared, leaving a clean precipitate of silver iodide. It was similarly found that Hammarsten's "Casein" gave the reaction, after

having been first carefully extracted with ether to make sure of the absence of fat. These results seemed to point to the possibility of the occurrence in protein of methylated nitrogen. The reaction could not be due to methoxy derivatives, as the precipitate of silver iodide was only formed when the reaction mixture was heated to about 230°.

To investigate this further, a dried pancreatic digest of protein, also carefully extracted with ether, was examined. This too gave a positive result, as did also a phosphotungstic precipitate from the digest, and the filtrate from the precipitation. It seemed desirable at this stage to make sure of the soundness of the reaction when applied to individual known amino-acids. Instead of the negative results expected, it was found that a mixture of glycine, tryptophane and a leucine-valine fraction gave a silver iodide precipitate. Of these separately, glycine and the leucine-valine fraction were positive, whilst tryptophane was negative.

On the basis that 100 grams of silver iodide correspond to 6·38 grams of methyl, the following percentages of methyl (CH_3) present in combination with nitrogen were found:

Glycine	0·34 %
	0·46
Alanine	0·43
	0·63
Leucine-valine	0·68
	0·95
Histidine	0·03
Tyrosine	negative
Tryptophane	negative
Commercial gelatin ...	0·77 %
Hammarsten's casein ...	0·97

In the case of the amino-acids the explanation seems to be that after the hydriodic acid has been distilled away, during the dry heating at 250°–270°, simple amino-acids break up, giving off carbon dioxide and leaving an amine, which should quite properly give the reaction. Thus glycine would decompose as follows:

$$NH_2.CH_2.COOH = NH_2.CH_3 + CO_2.$$

Such an explanation would seem to apply even to leucine. If it broke up in this way, isoamylamine would be formed; and in fact when isoamylamine was tested by the reaction, a silver iodide precipitate was formed in amount corresponding to the above percentages.

It will be noticed that the values for protein are slightly greater than those for the individual amino-acids, and yet it is apparently only the amino-acids of simple constitution which do give a positive result. It may therefore

be argued that as these can only form a small fraction of the protein, the high values with protein are only partly explained. It is indeed possible that methylamino groupings do exist in protein, but this reaction does not afford a sufficiently reliable test for their presence. Quantitatively the limits of error are as wide as +3 % and −15 %. Thus sarcosine, which on the above considerations might be expected to give a higher value than the theoretical 16·8 % for methyl attached to nitrogen, actually gave 14·4 %. When it is seen that simple amino-acid groups are broken up at the high temperature of 250° and in the presence of hydriodic acid, the likelihood that there are other groupings in protein itself (not methylamino) which will also break up becomes extremely great.

The work out of which this note has arisen was begun during the tenure of the Michael Foster Studentship in Cambridge University. My thanks are due to Dr F. G. Hopkins for suggesting the work and for supervising its earlier stages.

REFERENCES.

Herzig and Meyer (1894), *Monatsh.* **15**, 613.
—— —— (1895), *Monatsh.* **16**, 599.
—— —— (1897), *Monatsh.* **18**, 379.
Trier (1913), *Zeitsch. physiol. Chem.* **85**, 383.