THE CHEMISTRY OF HEHNER'S TEST FOR FORMALDEHYDE IN MILK.

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Since Hehner's test for formaldehyde in milk was first described, a large amount of analytical work has been published on the subject. Nevertheless very little attention has been directed towards the elucidation of the chemistry of the reaction.

While studying the nature of the Adamkiewicz reaction with acetic acid for proteins,* I was led to investigate their behaviour with formaldehyde, and I was

* The term "protein" is used instead of "proteid," in accordance with the proposals of the joint committee of the Chemical and Physiological Societies.

able to show that the presence of formaldehyde as an impurity in the acetic acid undoubtedly played an important part (O. Rosenheim, *Biochem. Journal*, 1906, **1**, 233). It was found that formaldehyde in the presence of strong sulphuric acid and an oxidizing agent gave with proteins generally the same purple-violet-coloured ring as does the impure acetic acid, showing, furthermore, the characteristic spectrum absorption band between D and E.

It thus became obvious that Hehner's test is an inverse instance of a general reaction of formaldehyde with proteins. The conclusions arrived at in the study of the latter are therefore directly applicable to Hehner's test, and may, perhaps, be referred to here, the more so as my publication has escaped the attention of those recently working on this subject (S. F. Acree, *Journ. Biol. Chem.*, 1906, **2**, 145; H. S. Shrewsbury, ANALYST, 1907, **32**, 5; F. W. Richardson, *Journ. Soc. Chem. Ind.*, 1907, **26**, 3).

Pure sulphuric acid and pure formaldehyde give no colour reaction with proteins, but the colour is obtained after the addition of small amounts of oxidizing substances,* such as ferric chloride, potassium nitrite, platinic chloride, sodium peroxide, hydrogen peroxide, potassium percarbonate, ammonium and potassium persulphate. The last is in many ways the most convenient, being a dry crystalline substance easily obtainable in a state of purity. The amount of oxidizing agent necessary is very small.

The reaction fails if the quantity of formaldehyde is increased beyond a certain limit, which is in proportion to the amount of oxidizing agent employed. When this limit is exceeded, the strong reducing power of formaldehyde towards oxidizing agents is exerted, and the occurrence of the reaction is prevented.

Three possibilities present themselves in regard to the action of formaldehyde on proteins in the presence of oxidizing agents:

1. The products of the oxidation of the protein interact with formaldehyde to produce the colour reaction. This possibility was excluded, for no colour was obtained when the protein was oxidized before adding the formaldehyde.

2. The formaldehyde is oxidized first, giving rise to an intermediate (formic acid, the final oxidation product, gave negative results) oxidation product, which reacts with the protein. Evidence that the reaction can take place in this manner was afforded by investigating the behaviour of the intermediate oxidation product of formaldehyde — Diformaldehyde - peroxide - hydrate ($OH.CH_2O.O.CH_2O.OH$). Although this substance is not readily obtainable in a pure state,† its ammonium compound has been prepared and described by A. Baeyer and V. Villiger (*Berichte*, 1900, **33**, 2479). It may be obtained as a white crystalline powder by the interaction of hydrogen peroxide and formaldehyde in ammonium sulphate solution. An aqueous suspension of this substance was found to react with proteins and *pure* sulphuric acid, producing the characteristic colouring.

3. Under the usual conditions of the reaction, the formaldehyde may combine

* W. B. Wherry (*Dept. of the Interior, Bur. of Gov. Lab.*, 1905, **31**, 17) has lately shown that certain preparations of Witte's "peptone" contain traces of nitrites. Such preparations will react with pure sulphuric acid and formaldehyde.

† It was first found among the products formed by slow oxidation of ether (L. Legler, *Berichte*, 1881, **14**, 602; 1885, **18**, 3343), and is also probably formed in ether exposed to light.

with the protein first, the resulting aldehyde-protein compound (F. Blum, *Z. f. Phys. Chem.*, 1896, **22**, 127; A. Benedicenti, *Arch. f. Anat. u. Phys.*, 1897, 219; L. Schwarz, *Z. f. Phys. Chem.*, 1900, **31**, 460) being subsequently oxidized. These two stages of the reaction, taking place concurrently under the second possibility (*loc. cit.*), can be demonstrated by preparing the pure colourless aldehyde-proteid compound, and submitting it to the action of an oxidizing agent and sulphuric acid. The casein-formaldehyde compound, prepared from pure casein (Hammarsten) and washed free from formaldehyde, was used. The characteristic colour reaction was obtained.

As already stated, the formaldehyde reaction is a general one for proteins, and it depends on the presence of the tryptophane-group in the protein molecule. Tryptophane has been isolated by Hopkins and Cole (*Journ. Physiol.*, 1901, **27**, 418. See also Neuberg and Popowsky, *Biochem. Zeit.*, 1907, **2**, 357) from the products of protein hydrolysis, and the constitution of an indole-amino-propionic acid is now ascribed to it by Ellinger (*Berichte*, 1906, **39**, 2515). Tryptophane prepared from casein shows the formaldehyde reaction. The intensity of the reaction with different proteins varies in direct proportion to the amount of tryptophane present in the protein molecule. The negative result given by gelatine is thus explained, for no tryptophane is obtainable from this substance (Hopkins and Cole, *loc. cit.*).

With indole and skatole, and other heterocyclic compounds, certain colour reactions are obtained, which will be described later. They may furnish a clue as to the nature of the colouring matter produced, which is at present under investigation.

The certainty with which the reaction is given by tryptophane and also by indole and skatole, etc., suggest the use of these compounds for the preparation of standard solutions in the colorimetric estimation of small quantities of formaldehyde.