A COLOUR REACTION OF FORMALDEHYDE WITH PROTEIDS AND ITS RELATION TO THE ADAMKIEWICZ REACTION

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More than a year ago I observed that the addition of a trace of formaldehyde to acetic acid, which had been deprived by distillation¹ of its power to give, with sulphuric acid, Adamkiewicz's² reaction for proteids, restored to it its chromogenic power in a very marked degree. As would be expected, it was found that the acetic acid was not essential, the formaldehyde, proteid and sulphuric acid giving by themselves an identical reaction. On increasing the quantity of formaldehyde the reaction failed after a certain percentage of it had been reached. The explanation of this apparent anomaly was found in the presence of small quantities of oxidising substances (nitrous acid, ferric salts) in the ordinary sulphuric acid employed. Pure sulphuric acid and pure formaldehyde³ produce no colour reaction with proteids, but after the addition of small amounts of oxidising substances to the acid the reaction is obtained, provided that the amount of formaldehyde is very small. When the amount is sufficiently increased, its strong reducing power towards oxidising agents is exerted and prevents the occurrence of the reaction.

Three possibilities present themselves in regard to the action of formaldehyde on proteids in the presence of oxidising agents: (1) The proteid is oxidised first, and its oxidation-product interacts with formaldehyde to produce the colour reaction. This possibility was excluded by

1. F. G. Hopkins and S. W. Cole, *Proc. Royal Soc.*, 68, p. 23, 1901.

2. Adamkiewicz, *Pflüg. Archiv.*, IX, p. 156, 1874; *Ber. d. d. Chem. Ges.*, VIII, p. 161, 1875.

3. Two samples of formaldehyde were met with which gave the reaction with pure sulphuric acid. This was no doubt due to their containing a trace of hydrogen peroxide (cf. A. Bach, *Moniteur Scient.* [4] XI, p. 479, 1897) or some other active impurity.

negative experiments with proteids treated preliminarily with oxidising agents; (2) The formaldehyde is oxidised first, giving rise to an intermediate oxidation-product which reacts with the proteid. Decisive evidence for the fact that the reaction can take place in this manner was given by the investigation of the behaviour of the intermediate oxidation-product of formaldehyde. This must be regarded as an aldehydic-peroxide (Diformaldehyde-peroxide-hydrate $\text{OH}\cdot\text{CH}_2\cdot\text{O}\cdot\text{O}\cdot\text{CH}_2\cdot\text{OH}$). This substance is not readily obtainable in a pure state¹, but its ammonium compound has been prepared and described by A. Baeyer and V. Villiger². It can easily be obtained absolutely pure 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 proteids and pure sulphuric acid, producing the same characteristic colour ring as is obtained in the Adamkiewicz reaction; (3) Under the usual conditions of the reaction the formaldehyde may combine with the proteid first, the resulting aldehyde-proteid compound (F. Blum,³ A. Benedicenti,⁴ L. Schwarz⁵) being subsequently oxidised. These two stages of the reaction, taking place concurrently under (2), can be demonstrated by the preparation of the pure, colourless aldehyde-proteid compound⁶ and its subsequent treatment with oxidising sulphuric acid. The characteristic colour reaction is obtained.

The following oxidising agents have been found to give the reaction in question in the presence of sulphuric acid and formaldehyde: hydrogen peroxide, potassium persulphate, sodium peroxide, platinum chloride, ferric salts, potassium nitrite. The amount of oxidising agent necessary is very small. Nearly every sample of

1. This substance was first found among the products formed by the slow oxidation of ether (L. Legler, *Ber. d. d. Chem. Ges.*, XIV, 1881, p. 602; XVIII, 1885, p. 3343.) It is extremely likely that it is also formed in ether exposed to light (Legler, *loc. cit.*), and it seems therefore not improbable that Liebermann's reaction (*Centralbl. d. med. Wiss.*, 1887, p. 321) for proteids after their treatment with active ether is due to the presence in the latter of this substance and not of glyoxylic acid, as supposed by S. W. Cole (*Journ. Phys.*, XXX, 1904, 311).

2. *Ber. d. d. Chem. Gesell.*, XXXIII, 1900, p. 2479.

3. *Zeit. f. Phys. Chem.* XXII, 1896, p. 127.

4. *Arch. f. Anat. u. Physiol.*, 1897, p. 219.

5. *Zeit. f. Phys. Chem.* XXI, 1900, p. 460.

6. The casein-aldehyde compound, washed completely free from formaldehyde, was the one made use of. If the aldehyde compounds are prepared according to Schwarz (*loc. cit.*) by means of alcohol and ether precipitation, it is here specially important to use ether free from peroxide and aldehyde, as otherwise these might give rise to Liebermann's reaction (see supra).

'coml.' sulphuric acid examined was found to give the reaction without needing the addition of an oxidising agent. The amount of formaldehyde necessary is also very small, 1 : 1,000,000 still giving the reaction.

For practical purposes a dilution 1 : 2500 (*i.e.*, 10 c.c. of the commercial 40 per cent. formaldehyde solution to 1 litre) has been generally used. It is preferable to use pure sulphuric acid, and add to it a small quantity of ferric chloride (5 mg. to 100 c.c.), or potassium nitrite (2 mg. to 100 c.c.), or of potassium persulphate, etc. A purple ring soon develops at the surface of contact.

The contact ring of the formaldehyde reaction is absolutely characteristic, and identical in appearance with that of the Adamkiewicz reaction. The colour varies, exactly as in the Adamkiewicz reaction according to the relative amounts of proteids and formaldehyde used from violet to purple. The absorption spectrum of the formaldehyde colouration is identical with that of the Adamkiewicz reaction, showing the same band between D and E.¹

The reaction with formaldehyde is a general one for proteids and depends, just as has been shown conclusively for the Adamkiewicz reaction by Hopkins and Cole,² on the presence of the tryptophane indole group in the proteid molecule. All substances which give Adamkiewicz or Hopkin and Cole's glyoxylic acid reaction also show the formaldehyde reaction. The following proteids have been examined with positive results: crystallised egg-albumin, crystallised serum-albumin, lact-albumin, caseinogen,¹² casein (Hammarsten), fibrin, Witte's peptone, hetero-, proto- and deuterio-albumoses, elastin, chondrin, vitellin, keratin, mucin, myosin. Two vegetable proteids, crystallised edestin (from hempseed) and gliadin (gluten), were examined and also gave the reaction, as did commercial preparations of the following enzymes: pepsin, trypsin, diastase, takadiastase. The

1. In preparing the solutions for spectroscopic observation it was found advisable to use a more dilute sulphuric acid than usual in order to prevent the formation of secondary products due to the effect of strong acid upon the proteid (cf. also Hopkins and Cole, *loc. cit.*).

2. *Journ. of Physiol.*, XXVII, 1901, p. 418.

3. It is here of interest to note that a test for formaldehyde in milk, introduced by O. Hehner, depends on the formation of a blue ring on the addition of sulphuric acid to formalised milk.

reaction is well shown by crystallised tryptophane (indole-amino-propionic acid) prepared by the method of Hopkins and Cole (*loc. cit.*).

No reaction is obtained with gelatin (absence of indole group). With indole and skatole a rose-red colour, unmistakably different from that obtained with proteids is produced.¹ With tyrosin, only a negative result has been obtained. Under the conditions of the reaction above described the green colour reaction of Denigés² is not observed.

The power to produce this colour reaction is limited to formaldehyde, the lowest member of the series. It is given as well by all those compounds which give rise to formaldehyde by the action of acids, such as para-formaldehyde, methylal, hexamethylen-tetramine, etc. All the other aliphatic aldehydes examined — acetic, propionic, amylic, oenanthicaldehyde—as well as chloral-hydrate—fail to produce the reaction. That aromatic aldehydes³ give rise to varying colour reactions with proteids under the influence of strong acids has been shown, by C. Reichl,⁴ S. W. Cole,⁵ and E. Rohde.⁶ These reactions are, however, although depending on the indole group of the proteids (cf. Cole, Rohde, *loc. cit.*), evidently of quite a different character to that of the one with formaldehyde.

THE RELATION OF THE FORMALDEHYDE REACTION TO THE ADAMKIEWICZ REACTION

The resemblance in colour, character and absorption spectrum of the formaldehyde reaction to the original Adamkiewicz reaction is striking and unmistakable. When we remember that formaldehyde is formed in considerable quantities by the oxidation of acetic acid

1. The investigation of the behaviour with formaldehyde of indole and its derivatives, as well as of other simple heterocyclic compounds of physiological interest is being continued. The results obtained will be communicated in a later paper, and will, it is hoped, furnish a clue to the real nature of the colouring matter produced in the Adamkiewicz reaction.

2. *Compt. Rend.*, CXXX, 1900, p. 583.

3. It is of interest that the reaction with aromatic aldehydes is obtained with *pure* sulphuric acid, a fact observed by me before Rohde's publication. I had found too that the addition of oxidising agents (impure H_2SO_4 , H_2O_2 , KNO_2 , etc.) has a marked influence on the reaction. That potassium nitrite exerts this influence has been stated by F. A. Steensma (*Zeit. f. phys. Chem.*, XLVII, 1906, p. 24) since this paper was written.

4. *Monatshefte f. Chem.*, X, 1889, 317, XI, 1890, 155.

5. *Loc. cit.*

6. *Zeit. f. Phys. Chem.*, XLIV, 1905, 161.

with hydrogen peroxide (Hopkins and Cole, *loc. cit.*), and that hydrogen peroxide is always present in acetic acid exposed to the action of light (Bach, *loc. cit.*), it seems an unavoidable conclusion that formaldehyde is a common impurity of acetic acid. This and the hydrogen peroxide as oxidising agent furnish the necessary conditions for the formaldehyde reaction, which must therefore be regarded as at any rate playing a part in the Adamkiewicz reaction. This conclusion is strengthened by the observation I have often had occasion to make that an acetic acid, which with pure sulphuric acid only gives a relatively slight Adamkiewicz reaction, shows a much more marked chromogenic power with sulphuric acid containing oxidising agents. It is interesting to note that an analogous observation was made as early as in 1888 by E. Salkowski,¹ who, however, offered no explanation for it. He found that the reaction is enormously increased by the addition of a 'minimal' amount of potassium nitrite, but that the opposite effect is produced by large amounts. Both my own and Salkowski's observations are readily explained when one remembers the importance in the formaldehyde reaction of a proper relation between the amounts of formaldehyde and oxidising substance. They show further that in regard to the Adamkiewicz reaction we have to consider not only impurities of the acetic acid but also those of the sulphuric acid.

It is a noteworthy fact that the power to produce the reaction is only exercised by the lowest member of the aliphatic aldehyde series. Hopkins and Cole, as is well known, consider that the lowest member of the aliphatic aldehyde-acid series—glyoxylic acid—is the only constituent in active acetic acid efficacious for the production of the Adamkiewicz reaction. Although from a theoretical standpoint it is extremely difficult to understand why the mere introduction of a carboxylic group should confer chromogenic properties on formaldehyde, the experimental evidence above brought forward does not exclude the possibility that glyoxylic acid, as well as formaldehyde, plays a part in the Adamkiewicz reaction. Once, however, the efficacy of formaldehyde has been established, the question inevitably arises

1. *Zeit. f. Phys. Chem.*, XII, 1888, 221.

whether glyoxylic acid acts as such or only as an intermediate product in the formation of the really active substance, formaldehyde. Hopkins and Cole hold that *pure* glyoxylic acid reacts with proteids in the presence of *pure* sulphuric acid. In spite, therefore, of the ease with which glyoxylic acid gives rise to formaldehyde¹ in the presence of oxidising agents, their reaction could, in the light of my results, in no way depend on the agency of formaldehyde. The methods employed by Hopkins and Cole, however, for the preparation of glyoxylic acid, do not absolutely exclude the presence and activity of oxidising agents. Even the calcium glyoxylate made use of in one case, may from the method of its preparation (from alcohol by means of nitric acid) have been contaminated with the per-acid salt. This is all the more likely if we remember the affinity of lime for hydrogen peroxide.² The preparation of their 'reduced oxalic acid' solution by means of sodium amalgam might seem to exclude the possibility of the presence of an oxidising agent, but if we consider what a small quantity of this is necessary and the way in which hydrogen peroxide is formed under the most unexpected conditions, it seems by no means impossible that even here an oxidising agent may have been present.³

A method of preparing glyoxylic acid which will exclude the presence and formation of an oxidising agent would seem to be required to settle the question, such a method for instance as the action of water on di-bromo-acetic acid or on di-chloro-acetic ethylester.

I may here mention that I have had three samples from different sources of 'pure' syrupy glyoxylic acid, none of which in freshly prepared solution showed the slightest reaction with proteids and pure sulphuric acid. Although the substances may not

1. Hopkins and Cole obtained from 1 litre of oxidised acetic acid 'considerable' quantities of the osazone of formaldehyde, whilst they only obtained 4 decigrammes of the osazone of glyoxylic acid from 3 litres of the same acetic acid. In all the cases where they obtained the Adamkiewicz reaction with the products of oxidation by means of hydrogen peroxide of various aliphatic acids and aldehydes, and drew the conclusion, without isolating glyoxylic acid, that it had been formed, it is most probable that formic aldehyde had also been produced and took part in the reaction. The substances oxidised by them with hydrogen peroxide were: tartaric, glycollic, glyceric, pyruvic, and paralactic acid, glycerin, glycollic aldehyde, and dextrose.

2. Cf. M. Traube, *Ber. d. d. Chem. Ges.* XXVI, 1893, p. 1471.

3. Hoppe-Seyler (*Zeit. f. Phys. Chem.* II, 1878, p. 22), showed that palladium-hydrogen in presence of water gives rise to hydrogen peroxide. Schönbein noticed long ago that lead amalgam acting in dilute H_2SO_4 produces hydrogen peroxide. M. Traube (*loc. cit.*) has demonstrated that nearly the theoretical yield of hydrogen peroxide is obtained by the action of zinc-amalgam on water.

have been *pure* glyoxylic acid, it was demonstrated that they contained it by various tests (reduction of silver and Fehling's solution, preparation of phenylhydrazin compound). The presence of some deleterious impurity may, of course, be assumed, but the solutions of the acids in question on keeping (exposed to light) became strongly active with pure sulphuric acid and proteids. A sample of glyoxylic acid prepared electrolytically from oxalic acid in sulphuric acid solution gave the reaction in question most markedly, but it is of importance here to remember that very powerful oxidising agents (persulphuric acid and hydrogen peroxide) are known to be produced in the electrolysis of sulphuric acid.¹ It is true that Hopkins and Cole declare that the presence of a trace of hydrogen peroxide suffices to prevent their glyoxylic acid reaction. But my own experience is not in accord with theirs on this point. It was found that if a sufficient amount of glyoxylic acid be present quite a large quantity of hydrogen peroxide can be added without preventing the reaction which does, however, cease when the hydrogen peroxide is in sufficient excess. We find here again the same relationship between the amounts of oxidising agent and aldehyde necessary for the occurrence of the reaction, which exists when formaldehyde itself is employed.

In conclusion it may be pointed out that not only is the formaldehyde reaction an absolutely reliable one, but that it possesses the further advantage of being carried out with a substance now readily procurable in commerce. The reaction has been used for more than a year in the practical classes of this laboratory in place of the somewhat uncertain Adamkiewicz reaction.

SUMMARY

1. Formaldehyde gives rise to a characteristic colour reaction with proteids in the presence of sulphuric acid containing oxidising agents.
2. The same reaction is produced by the interaction of di-formaldehyde-peroxide-hydrate with proteids and pure sulphuric acid.
3. This reaction is due to the formation of a proteid-formaldehyde compound and its subsequent oxidation.
4. The reaction depends on the presence of the Tryptophane (Indole) group in the proteid-molecule.

1. Berthelot, Traube, Baeyer, and Villiger (*Ber. d. d. Chem. Ges.*, XXXIV, 1901, p. 859).

5. The colour obtained is identical spectroscopically with that produced in the Adamkiewicz reaction and in Hopkins and Coles' glyoxylic acid reaction.

6. The Adamkiewicz reaction is not only due to impurities of the acetic acid used, but is influenced by the presence of oxidising agents in the sulphuric acid (nitrous acid, ferric salts, etc.)

7. The question is discussed how far the Adamkiewicz reaction is due to the presence or formation of formaldehyde in the acetic acid used.

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