CLIV. A COLOUR REACTION FOR DISULPHIDES.

By ERNEST WALKER.

From the Department of Biochemistry, Oxford.

(*Received October 19th, 1925.*)

THE colour reaction here to be described was discovered in the course of some work upon the sulphur constituents of tissue. Reference to the literature, however, revealed that the reaction had been applied previously in the specific case of cystine. As the reaction appears to be capable of wide application and is simple to carry out, it is proposed to give some account of it here and to mention a few of its applications.

The actual test is simply a modification of the well-known nitroprusside reaction for the sulphydryl group, a solution of potassium (or sodium) cyanide being used instead of ammonia or other alkali. In the case of a solution containing a disulphide, a few drops of 5 % aqueous sodium nitroprusside are added, followed by 3–5 drops of 10 % aqueous potassium cyanide. The final reaction of the solution must be alkaline. If the disulphide is present in high concentration, the colour, a deep magenta, develops immediately; if present in low concentration, there is a delay of a few minutes. For example, an alkaline solution of cystine, containing 1 part in 1000, shows a slight delay and the colour may not reach its maximum intensity for 20 minutes. A solution containing 1 part in 10,000 shows a somewhat longer delay and the final coloration is only a faint pink. This concentration (1 : 10,000) about represents the limit of sensitivity of the reaction. In the examination of tissues the test may be performed in the presence of a high concentration of ammonium sulphate, as recommended by Hopkins for the sulphydryl test. In all cases the colour remains stable for an hour or longer. It is important to note that the reagents used will give a similar colour with the —SH group, if such be present; this may be controlled by first testing with sodium nitroprusside and ammonia. Furthermore, the slight delay which occurs in the disulphide reaction serves to differentiate it from the instantaneous colour given by sulphydryl compounds.

With regard to the mechanism of the reaction, there can be little doubt that the cyanide effects the reduction of the disulphide grouping to the sulphydryl grouping and that the latter then gives the normal nitroprusside reaction. Mauthner [1912] showed that cystine is reduced to cysteine by aqueous potassium cyanide, and more recently Abderhalden [1923] has made use of this reducing power of cyanide in investigating the oxidation of cysteine.

Sullivan [1925], in describing a specific colour test for cysteine, states that this colour reaction is given by cystine in the presence of sodium cyanide, owing to the reduction of the cystine to cysteine by the cyanide.

The following is a brief description of a number of miscellaneous applications of the disulphide colour reaction.

In the first place, all disulphides tested have given a positive reaction. These are oxidised glutathione, cystine, dithiodiglycol[1], dithiodiglycollic acid and diethyl disulphide. With regard to the application of the test to tissues, it has been possible to demonstrate the presence of a disulphide grouping (presumably due to cystine) in hair, nails and the horny layer of skin. The hair used was that from a piece of white calf-skin and it was necessary to soak it in dilute acid in order to obtain a distinct reaction. The test has also been applied successfully to tissue (skin and water-extracted muscle) in which the sulphydryl group had previously been oxidised by hydrogen peroxide.

One case in which the colour reaction proved of use is described in the paper which follows this.

Applied to normal urine the test usually gives a faint positive reaction, corresponding to a disulphide concentration of 1 in 10,000. At such a dilution no quantitative significance is attributed to the test.

Harris [1923] has shown that native ovalbumin gives no sulphydryl reaction, but that on denaturation it gives a vivid reaction. He suggests that the precursor of the —SH formed on denaturation is not a —S—S grouping. The application of the disulphide reaction to native ovalbumin gives a negative result, thus substantiating Harris's suggestion.

Blood serum gives no —SH reaction, either in the native state or after coagulation. In the native state, serum gives only a very faint disulphide reaction, such as is given by a disulphide concentration of 1 in 10,000, although some two-thirds of its total sulphur is supposed to exist in the form of cystine. On the other hand, serum gives a vivid disulphide reaction after coagulation by heat. This change is thus analogous to that which occurs in ovalbumin, reading —S—S— for —SH, and is in agreement with the recent observation of Hopkins [1925] that thiol compounds react with serum proteins only after denaturation. What the precursor of the disulphide group is, it is difficult to say, but the facts suggest that the disposition of the cystine moiety in serum albumin is not simple.

Summary.

A colour reaction for the detection of the disulphide grouping is described. The reagents used are potassium cyanide and sodium nitroprusside.

A few illustrations of the applicability of the colour test are briefly described.

[1] It was observed that this compound is readily reduced, both aerobically and anaerobically, by water-extracted muscle powder. Being neutral in reaction it should prove more serviceable than thioglycollic acid in the investigation of biological oxidations.

Harris's suggestion, that the precursor of the sulphydryl group formed on denaturation of ovalbumin is not a disulphide linkage, is confirmed. It is also shown that blood serum in the native state gives no disulphide reaction, but gives a vivid reaction after coagulation.

I wish to record my thanks to Professor Peters for his interest and advice.

REFERENCES.

Abderhalden (1923). *Pflüger's Arch.* **198**, 122.
Harris (1923). *Proc. Roy. Soc. Lond.* B. **94**, 426.
Hopkins (1925). *Biochem. J.* **19**, 787.
Mauthner (1912). *Z. physiol. Chem.* **78**, 28.
Sullivan (1925). *Abstracts Bact.* **9**, 37.