INFLUENCE OF COPPER AND EDTA ON THE ALKALINE OXIDATION OF ADRENALINE

By J. G. Lennart Harthon

From the Analytical Department, Research Laboratories, AB Bofors, Nobelkrut, Bofors, Sweden

Received April 15, 1959

Adrenaline can be determined quantitatively in the presence of sulphite by measuring the maximum fluorescence obtained by oxidation in alkaline solution. The presence of copper in the solution quenches the fluorescence according to Stern-Volmer's law. Ethylenediaminetetra-acetic acid present in copper-free solutions has no influence on the fluorescence intensity curve. When copper is present as EDTA-complex the oxidation is strongly catalysed and the fluorescence maximum appears earlier the higher the concentration of complex. The reactions do not seem to be photochemically influenced.

Adrenaline in low concentrations can be quantitatively determined by measurement of the transient fluorescence from an unstable intermediate product, *N*-methyltrihydroxyindole, formed during the alkaline oxidation of adrenaline[1,2].

Copper ions are known to catalyse the oxidation of adrenaline[3,4] and so it is necessary to keep the concentration of copper as low as possible in pharmaceutical preparations containing adrenaline. Green, Mazur and Shorr[5] have studied the effect of iron on the oxidation of adrenaline at pH 7·4 and showed that this catalysed oxidation is accelerated tenfold in the presence of the chelating agent ethylenediaminetetra-acetic acid (EDTA).

The purpose of this preliminary investigation was to study how the presence of copper influenced the fluorescence obtained in the determination of adrenaline based on its oxidation in strongly alkaline solution and to what extent the simultaneous presence of EDTA changed the fluorescence-time relation. As the primary interest in this reaction was its use in the quantitative fluorimetric determination of adrenaline in anaesthetic solutions, all the adrenaline solutions also contained 0·05 per cent sodium metabisulphite. This means that the copper might be present in the monovalent state, either as a complex with sulphite $(CuSO_3)^-$ or hydroxide as experiments indicated that there was still sulphite present, even after the adrenaline was oxidised.

Experimental

All chemicals were selected to contain a low content of heavy metals. The water used was repeatedly distilled in all-glass apparatus and its copper content was less than 2 μg./l. Stock adrenaline solutions were prepared by dissolving adrenaline base (UCLAF, Paris) and sodium metabisulphite in diluted hydrochloric acid. The final working solutions were prepared so that they were 0·01 N in hydrochloric acid and contained 0·05 per cent sodium metabisulphite.

The alkaline solution for the oxidation consisted of 1·1 N sodium hydroxide in 50 per cent (v/v) ethanol with the content of oxygen which was obtained when the solution was equilibrated in air.

The mixture on which the fluorescence was measured was made from 20 ml. of oxidation solution to which was first added 3·00 ml. of water or the same amount of a solution containing the appropriate amounts of copper sulphate and EDTA, and then, rapidly, 2 ml. of adrenaline solution. Time was measured with a stop watch when the adrenaline solution had been added. The first reading was made 15 seconds later and then readings were taken every 10 seconds for about 6 minutes. The blank value of the solution was taken from the same solution about an hour later when fluorescence had ceased.

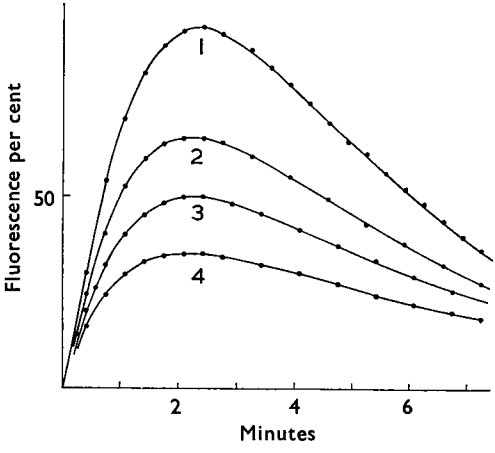

FIG. 1. Fluorescence-time relation for the alkaline oxidation of 0·8 μg./ml. of adrenaline (1). The same reaction for solutions containing in addition 0·08 μg./ml. of Cu (2); 0·2 μg./ml. of Cu (3); and 0·4 μg./ml. of Cu (4).

The fluorescence was measured in a Photovolt filter-fluorimeter Model 540 with photomultiplier attachment and a Hanovia S4 mercury vapour lamp as a source for the exciting radiation. The primary filter had its transmission maximum at 365 mμ and the secondary filter at 505 mμ. The fluorescence standard consisted of a solution of 2-ethoxy-6,9-diamino-acridinelactate (Rivanol).

RESULTS AND DISCUSSION

For adrenaline solutions containing up to 0·8 μg./ml. of adrenaline, the maximum fluorescence showed good proportionality against concentration. Increasing the metabisulphite content to 0·5 per cent had no influence on the results. The time from mixing the solutions until maximum fluorescence was reached was about 135 seconds and independent of the concentration of adrenaline.

Figure 1 shows the relation between fluorescence and time for 0·8 μg./ml. of adrenaline with an additional amount of copper increasing from 0·0 to 0·4 μg./ml. It is apparent that the addition of copper lowers the maximum fluorescence (I_0) for a given amount of adrenaline. A linear relation between fluorescence (I) and inhibitor concentration (Q) cannot be demonstrated but if I_0/I is plotted against Q (Fig. 2) a straight line is obtained for a copper concentration of up to 1·2 μg./ml. This indicates that Stern-Volmer's law $I/I_0 = 1/(1 + KQ)$ is obeyed and, calculated in molar quantities from the slope of the curve, $K = 2{\cdot}4 \times 10^5$

$1 \times$ mol.$^{-1}$ is obtained. The fulfilment of Stern-Volmer's law indicates that copper in this reaction acts as a fluorescence quencher.

The time after which fluorescence maximum is reached seems to be independent of the amount of copper added (Fig. 1). But the experimental arrangements did not give sufficient accuracy in the fluorescence-time relation to permit any conclusions about the catalytic activity of copper on the oxidation. This is to be the subject for further investigations.

The effect of an addition of EDTA in concentrations up to 8 mg./ml. was tested on copper-free adrenaline solutions. No influence was noted on either the maximum fluorescence or the progress of the fluorescence. The results are, however, different if copper is present too. Figure 3 shows the changes in the progress of fluorescence of an adrenaline solution containing copper at different concentrations of EDTA. The maximum fluorescence appears earlier and is increased in comparison with the same solution without EDTA. Additions of EDTA up to 8 mg./ml. were investigated on a solution containing 0·8 μg./ml. of adrenaline and 0·2 μg./ml. of Cu. The highest fluorescence was obtained at about 2 mg./ml. of EDTA and was about 85 per cent of the fluorescence for a copper-free solution. At higher concentrations of EDTA the maximum fluorescence was again slowly diminished.

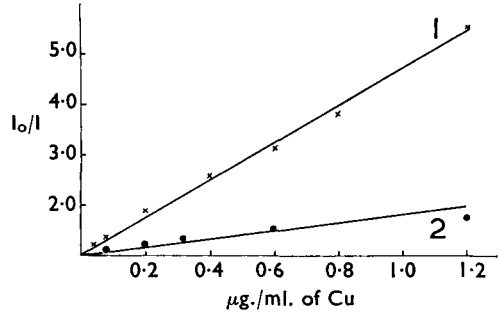

FIG. 2. Influence of increasing amounts of copper on the maximal fluorescence of adrenaline solutions (0·8 μg./ml.) containing (1) none or (2) 2 mg./ml. of EDTA.

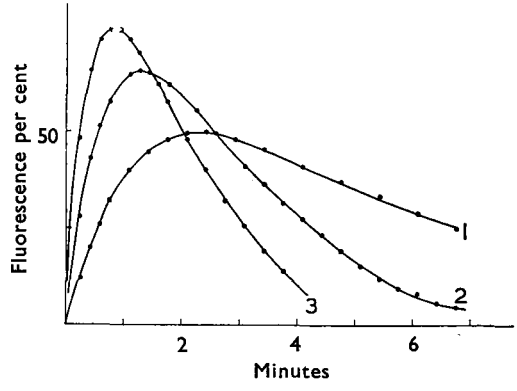

FIG. 3. Fluorescence-time relations for 0·8 μg./ml. of adrenaline and 0·2 μg./ml. of Cu containing (1) none; (2) 0·2; (3) 0·8 μg./ml. of EDTA.

The influence on the maximum fluorescence of different concentrations of copper at a constant content of EDTA (2 mg./ml.) is represented in Figure 2 and plotted in the same way as for EDTA-free solutions. It is evident that an increasing copper concentration decreases the fluorescence but not to the same extent as for EDTA-free solutions. The time for reaching fluorescence maximum is also shortened when copper and

EDTA are present together. In this experiment the following figures were obtained:

μg./ml. Cu	0·0	0·2	0·4	0·8	1·6
time for max in seconds ..	135	85	55	35	25

If it is supposed that the reaction products of these experiments are the same it is evident from Figure 3 that the simultaneous presence of copper and EDTA strongly catalyses the oxidation of adrenaline in alkaline solution. As the oxidation of adrenaline to adrenochrome is the rate determining reaction in the formation of the fluorescent substance[2], it is evident that this reaction is catalysed as the fluorescence maximum appears earlier. Figure 3, however, also indicates that the destruction of the fluorescent substance might be catalysed. The same phenomenon was observed by Ehrlén[6], when using this method for the determination of adrenaline in procaine solutions, and might have been due to some metal complex in his solutions. The reaction rates in these reactions will, however, be studied in more detail.

As copper can act as a photochemical catalyst, there was reason to see if the oxidation of adrenaline in these experiments was influenced in any way by the energy of the exciting radiation. This was performed in such a way that several fluorescence-time relations for solutions containing copper as well as copper-EDTA were recorded with different entrance slits on the fluorimeter. The slit was changed so that the fluorescence standard gave the same instrument deflection at a ten times lower sensitivity on the detector. In no case could any change in the course of the curves be noted. The oxidation of adrenaline under these conditions seems therefore not to be photochemically influenced.

References

1. Ehrlén, *Farm. Revy*, 1946, **45**, 753.
2. Heller, Setlow and Mylon, *Amer. J. Physiol.*, 1950, **161**, 268.
3. Chaix, Chauvet and Jezequel, *Biochem. Biophys. Acta*, 1950, **4**, 471.
4. Mørch, *Pharm. Weekbl.*, 1958, **93**, 141.
5. Green, Mazur and Shoor, *J. biol. Chem.*, 1956, **220**, 237.
6. Ehrlén, *Farm. Revy*, 1948, **47**, 242.