

## Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

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### The Formation of Hydrogen Peroxide During the Autoxidation of Ascorbic Acid

Ascorbic acid (Vitamin C) is usually described as a reducing agent, but several instances are known where it appears to act as an oxidizing agent. It has been shown that naphthalene is oxidized in aqueous acetone solutions of the vitamin<sup>1</sup>, whilst under similar conditions, anthracene, phenanthrene and 3:4-benzopyrene are converted to the corresponding quinones<sup>2</sup>. The mechanism of these oxidations appears to be unknown but it has been suggested that hydrogen peroxide formed during autoxidation of the ascorbic acid is involved in the process. Since it has now been shown that 3:4-benzopyrene can be directly oxidized with hydrogen peroxide to products which include 8:0 H benzopyrene and the 5:8-quinone<sup>3</sup> the above explanation would appear more feasible. As there appears to be no record in the literature of the proof of peroxide formation in the course of reactions involving ascorbic acid further evidence on this point has been sought.

Two tests for hydrogen peroxide have been used:—the formation of yellow pertitanic acid from titanil sulphate, and the formation of blue colour in the presence of ether, potassium dichromate and sulphuric acid. Both tests gave strongly positive results a few minutes after shaking ascorbic acid crystals with glass distilled water. Control tests of the water were negative. The possibility that the hydrogen peroxide found in the above experiments was, in any way, related to the addition of the test agents was eliminated by a further experiment. Ascorbic acid crystals were stirred into distilled water and after ten minutes the solution was shaken with ether; this being a reagent in which hydrogen peroxide, but not ascorbic acid, is soluble. After separation of the two layers some of the ether was withdrawn with a pipette and shaken with titanil sulphate solution. The development of a yellow colour showed the presence of hydrogen peroxide. The ether used in this experiment had previously been tested and found free of contaminant peroxide.

It is evident from the above that ascorbic acid in solution will readily give rise to hydrogen peroxide. Whether a catalyst is essential for the reaction is uncertain. The experiments described were carried out in scrupulously clean glassware with reagents of the highest possible purity, but the possibility of contamination with traces of metal or other agent cannot be completely excluded.

These findings offer an explanation of the ability of ascorbic acid to act as an oxidizing agent under certain conditions and additionally may explain the anomalous results which have been obtained in electrode potential determinations<sup>4</sup>.

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### Zusammenfassung

Es konnte gezeigt werden, daß in Lösungen von Ascorbinsäure freies Wasserstoffsuperoxyd entsteht. Hiermit läßt sich wohl erklären, daß derartige Lösungen unter bestimmten Bedingungen oxydierend wirken können.

### Photoreactivation in the near Ultra-violet of D-Glyceraldehyde-3-Phosphate Dehydrogenase

Partial reactivation of a crystalline preparation of triosephosphate dehydrogenase by irradiation in the near ultra-violet was observed during the course of an investigation of the inactivation of this enzyme by ultra-violet light and a measurement of the quantum yield for inactivation at 2537 Å.U.

The enzyme was prepared according to the method of CORI *et al.*<sup>1</sup> and recrystallized three times. In this form the enzyme has been shown to be firmly combined with a definite quantity of coenzyme (DPN) in the ratio of 1 mole DPN to 50,000 g triosephosphate dehydrogenase (TPD); neither recrystallization nor dialysis against distilled water remove the DPN<sup>2</sup>. CORI *et al.*<sup>3</sup> have recently shown that the DPN remains bound to the enzyme even after it has been reduced enzymatically in the presence of arsenate and glyceraldehyde phosphate; the method employed consisted of precipitation of the enzyme with ammonium sulphate following reduction of the DPN. During an independent study conducted at the same time it was found that reduced DPN (DPNH) could be readily dialyzed away against distilled water. The following test, involving no chemical alteration of the medium was therefore carried out: After reduction of the DPN in a 3 mg/cm<sup>3</sup> sample of the enzyme in pyrophosphate buffer at  $p_H$  8.5, 0.7 cm<sup>3</sup> was centrifuged for 2 hours at 60,000 rpm in a Spinco ultracentrifuge using the analytical cell divided into two equal compartments by means of a diaphragm perpendicular to the radial axis of the cell, the purpose of which is to prevent mixing at the end of the run. The two fractions were then removed and their DPNH contents measured spectrophotometrically by means of the system pyruvate-lactic dehydrogenase. The optical densities at 3400 Å.U. of 5-times diluted samples of the inner and outer fractions and of a non-centrifuged sample were, respectively, 0.022, 0.050, 0.060. The fact that not all the DPNH is accounted for in the two fractions was due to deposition of some of the enzyme at the bottom of the cell. How-

<sup>1</sup> W. P. JORRISSSEN, *Naturw. Tijdsche.* 19, 15 (1937).

<sup>2</sup> F. L. WARREN, *Biochem. J.* 37, 338 (1943).

<sup>3</sup> G. CALCUTT, *Brit. J. Cancer*, 4, 254 (1950).

<sup>4</sup> L. F. HEWITT, *Oxidation-Reduction Potentials in Bacteriology and Biochemistry* (Livingstone, Edinburgh, p. 60, 1950).

<sup>1</sup> G. T. CORI, M. W. SLEIN, and C. F. CORI, *J. Biol. Chem.* 173, 605 (1948).

<sup>2</sup> J. F. TAYLOR *et al.*, *J. Biol. Chem.* 173, 619 (1948).

<sup>3</sup> C. F. CORI, S. F. VELICK, and G. T. CORI, *Biochim. Biophys. Acta* 4, 160 (1950).