reasonable to suppose that an enzyme, which reversibly oxidizes diphosphothiamine to the disulphide, is present in baker's yeast.

The biological significance of the diphosphothiamine disulphide–diphosphothiamine equilibrium, which has also been studied polarographically[5] and the meaning of the occurrence of the disulphide in baker's yeast deserve further delucidation.

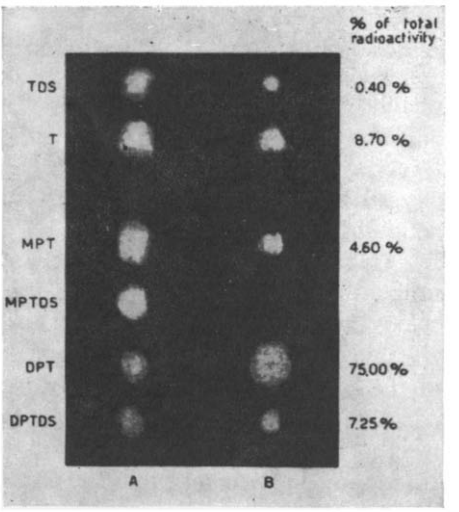

Fig. 1. Chromatogram of baker's yeast thiamine and thiamine disulphide compounds according to GREGOLIN *et al.*[1]. (A), reference mixture. (B), thiamine and thiamine disulphide derivatives from baker's yeast. T, thiamine; TDS, thiamine disulphide; MPT, monophosphothiamine; MPTDS, monophosphothiamine disulphide; DPT, diphosphothiamine; DPTDS, diphosphothiamine disulphide. The radioactivity of each spot has been measured by direct scanning of the paper using an end-window Geiger counter. The photograph of the chromatogram was taken in ultraviolet light at 254 mμ wavelength.

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The influence of some sulfur compounds on the catalysis by o-iodophenolate of ascorbate oxidation by oxygen

The bulk of information concerning the kinetics of ascorbate oxidation by molecular oxygen is based on catalysis of this process by heavy metal ions. In such systems many sulfur compounds of biochemical interest function as inhibitors by inactivating the heavy-metal ions by formation of a complex or an insoluble salt. In a preceding paper[1] we described another system with 7-iodo-8-hydroxychinoline-5-sulfonic acid (Ferron)

as a strong catalyst for the aerobic oxidation of ascorbate. A detailed study of the reaction mechanism revealed that neither stable oxidation–reduction carrier derived from the molecule of Ferron, nor contaminating metal chelate are responsible for the catalytic effect observed; a reaction scheme with a free radical of the Ferron phenolate ion as the catalytically active species of the system seemed to be most compatible with the experimental results. The catalytic activity of Ferron is shared by other iodophenols such as 3,5-diiodotyrosine and thyroxine, and the reaction mechanism seems to be very similar, being closely related to the *o*-iodophenolate configuration.

Based on the MICHAELIS[2] stepwise oxidation–reduction hypothesis, the incompletely reduced forms of oxygen are regarded as the true oxidizing agents for the oxidation of reduced components of the system, *i.e.* for ascorbate to its dehydro forms, and for Ferron to its free radical. The ascorbate–Ferron–oxygen system, where oxygen free radicals are generated, may be a model system, useful to the study of action of the compounds presumably interfering with similar systems *in vivo*.

Our attention was led to some sulfur compounds known to interfere with the utilization of iodine in the thyroid gland: acetylthiourea, methylthiouracil and benzthiooxazolidone. Thiocyanate was also included in this group. Oxygen analogues of the thio compounds, acetylurea, uracil and cyanate, were used to determine the effect of the sulfur atom itself, and glucothiooxazolidone was chosen as an analogue for benzthiooxazolidone from another point of view. The primary question we posed was, whether these compounds enter into the catalysed oxidation chain, since it is known that many sulfur compounds are easily able to form thyile free radicals[3], in suitable systems active as oxidation–reduction carriers. Their interaction would support the hypothetical free-radical mechanism of the Ferron-catalysed oxidation of ascorbate.

Representative results are given in the figures. Fig. 1 shows a comparison of oxidation rates of ascorbate in the absence and presence of Ferron and/or methylthiouracil (Alkiron) and their combinations at pH 7.0 and 8.0. In Fig. 2 the dependence of the catalytic activity of Ferron and thiocyanate on pH is shown, and in Table I the activities of all compounds tested are compared. These experiments were carried out with 0.5 m*M* ascorbate; qualitatively similar activation phenomena were

TABLE I

CATALYTIC ACTIVITIES OF VARIOUS SULFUR COMPOUNDS AND OXYGEN ANALOGUES

The experimental conditions are the same as under Fig. 1 (pH 7.0). The activities are expressed as multiples of the oxidation rate of ascorbate in presence of 0.5 m*M* Ferron.

Compound	*Catalytic activity*	*Concentration (M)*
KCNS	2.50	5.10^{-3}
KCNO	0.97	5.10^{-3}
Thiobenzooxazolidone*	2.60	5.10^{-3}
Thioglukooxazolidone*	0.96	5.10^{-3}
Alkiron	1.60	10^{-4}
Alkiron	1.83	5.10^{-3}
Uracil	0.91	10^{-3}
Acetylthiourea	1.80	10^{-3}
Acetylurea	1.02	5.10^{-3}

* These compounds are unstable. The stock solutions were prepared from the material freshly recrystallized from ethanol. In the course of the reaction the benzo-derivative forms a catalytically active system.

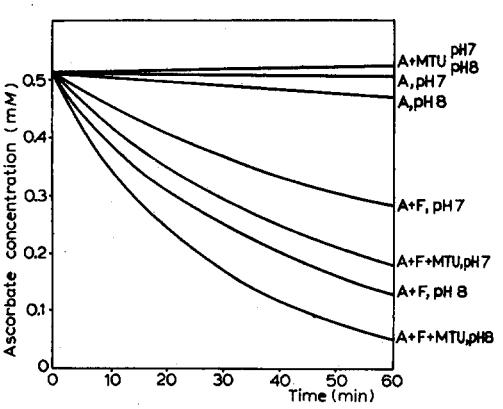

Fig. 1. The effect of Ferron and Alkiron on the autooxidation of ascorbate. Erlenmeyer flasks (100 ml) containing 20 ml reaction mixture with final concentrations of 0.5 m*M* Ferron, 0.1 m*M* Alkiron and about 0.5 m*M* neutralized ascorbate were shaken with air at 25°. At appropriate time intervals 2-ml samples were pipetted into a solution containing 6 ml water and 2 ml 5% HPO_3 and titrated with 0.5 m*M* 2,6-dichlorophenolindophenol solution standardized against $(NH_4)_2Fe(SO_4)_2 \cdot 7H_2O$. The reaction mixture was buffered to a final concentration of 0.1 *M* with $Na_4P_2O_7$ brought to the desired pH with redistilled HCl. All solutions were made from water redistilled from an all-glass apparature.

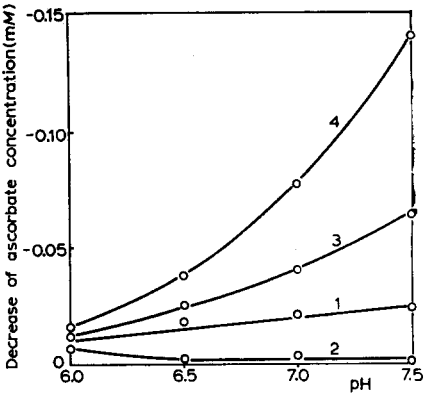

Fig. 2. The stimulating activity of thiocyanate on the Ferron-catalyzed oxidation of ascorbate as a function of pH. The conditions were the same as in Fig. 1: 1, 0.5 m*M* ascorbate; 2, ascorbate + 5 m*M* thiocyanate; 3, ascorbate + 0.5 m*M* Ferron; 4, all components. The activity is expressed as decrease of the ascorbate concentration after 60-min incubation.

Abbreviations: A, ascorbate; F, ferron, MTU, methylthiouracil.

obtained by the conventional Warburg manometric technique with 0.05 *M* ascorbate in pyrophosphate buffers.

The results presented show that, in appropriate concentrations, all sulfur compounds tested promoted the Ferron-catalysed oxidation of ascorbate. This activity is typically dependent on the catalytic activity of Ferron itself: if no Ferron was present or below pH 6.5, where the Ferron is not active, the sulfur compounds have only the known "protecting" effect on the ascorbate, in further lowering the very low oxidation rate in absence of the catalyst. It is obvious that the stimulating activity is confined to the sulfur function in the molecule, since the oxygen analogues of the active compounds are inactive in the system tested.

As to the mechanism involved in the stimulating effect of certain sulfur compounds we are aware that a more profound analysis of the reaction kinetics is needed for a conclusive interpretation of our results which up to now are only qualitative. But, on the basis of the previous extensive study of the *o*-iodophenolate catalysis[1], we are inclined to believe that a mechanism of the type proposed in the Fig. 3 is operating. In the reaction mixture the active forms of oxygen would be formed in the oxidation chain reactions, derived from the incompletely reduced oxygen molecules of a free-radical nature. When a compound able to form relatively stable free radicals is present, it may enter into the reaction chain by carrying the positive charge of the active oxygen to the donor, in the case of sulfur compounds the carrier system being composed of the thyile radical as the oxidized species and the nominal compound as the reduced species of the system. The stimulating effect would be caused by inhibition of the chain-terminating reaction, the recombination of oxygen radicals to H_2O_2.

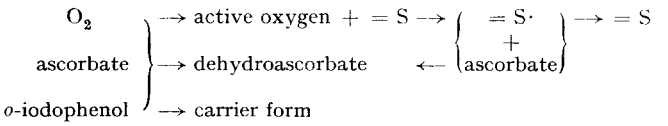

Fig. 3.

Apart from the fact that the sulfur atom in the molecule is the active site for the stimulating effect, the difference of catalytic activity of the pair benzthiooxazolidone and glucothiooxazolidone supports the view proposed, for the former should have in the conjugated system of the benzene nucleus a stabilizing effect for the thyile radical and correspondingly a higher activity in the iodophenolate system.

This scheme may be of general significance for the explanation of various activation and inhibition phenomena in systems where oxygen free radicals are generated, and two or more oxidizable donors are present. The activation or inhibition of the oxidation of one of them may be primarily determined by the position of their redox (or critical) potentials on the redox scale, if other factors for the formation and stability of free radicals are favorable.

Some enzyme systems are known for which the intermediary formation of oxygen free radicals is rather directly proved: for example, the classical preparation of heart-muscle cytochrome oxidase, which under conditions favorable for the slow reduction of oxygen forms benzopyrene quinones from 3,4-benzopyrene by cooxidation[4], or the systems composed of peroxidase and oxygen with additional activators studied in a recent series of papers by KLEBANOFF[5,6]. Among these activators thyroxine and other *o*-iodophenolic compounds function as activators for the oxidation of reduced pyridine nucleotide or sulfite; in our opinion, these systems bear a functional similarity with the Ferron- or thyroxine-catalysed oxidation of ascorbate, and also the explanation of the activation mechanism is based on similar assumptions as ours.

In a recent paper[7] the catalytic activity of several synthetic ligands, such as ferroin and phenanthroline, in the autooxidation of ascorbate is reported. From the comparable data it seems that different reaction mechanism from that involved in Ferron catalysis is involved, but more extensive comparison is necessary to be certain of this.

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