A note on free radical formation in biologically occurring quinones*

The concept that certain biological oxidation-reductions may proceed via successive single-electron transfers with the formation of free radical intermediates, has received considerable attention since the work of MICHAELIS[1]. The direct observation of these free radical intermediates has until recently[2,3,4], been quite difficult experimentally and only a relatively few reactions of biochemical interest have been studied directly utilizing the paramagnetism of these free radicals.

Paramagnetic resonance methods simplify and extend the sensitivity of these observations, and the free radical semiquinones of a number of biologically occurring quinones have recently been observed in this laboratory. This preliminary note is concerned principally with the qualitative behavior of these semiquinones, the kinetics of which continue under study.

It has been tentatively concluded on the basis of the paramagnetic resonance study of approximately 30 quinones that the non-enzymic oxidation-reduction of these compounds proceeds for all practical purposes, through two successive single-electron transfers as the molecule proceeds from its initial to its final state. The life time of the paramagnetic, semiquinone free radical appears to be determined in the first place by the isomeric form, the *para*-semiquinone being considerably more stable than the *ortho*, (in the case of naphthoquinones by a factor of perhaps as much as fifty) in agreement with the observed differences in oxidation potential. It has been found also that the free radical stability depends upon pH in such a manner that the semiquinone resulting from the dehydrogenation of a hydroxyl group is stabilized in alkaline solution, while the free radical formed by the dehydrogenation of an amino group is stabilized in acid solution. Thus the alkaline oxidation-reduction of the following compounds has been observed to produce a free radical concentration that may persist for as much as hours or days: *p*-benzoquinone, *o*-benzoquinone, adrenaline, DOPA, pyrogallol, gentisic acid, homogentisic acid, tocopherol, 1,4-naphthoquinone, 1,2-naphthoquinone, vitamin K_1, menedione, phthiocol, anthraquinone, anthraquinone-β-sulfonic acid, alizarin, carminic acid, and phenanthrenequinone. Carminic acid offers at least two modes of semiquinone formation so that a free radical is observed on either the oxidation or reduction of the original molecule. Of the aromatic amines, *p*-aminophenol and *p*-phenylenediamine have been observed to form semiquinone type intermediates with a stability increased in acid solution. In this respect they behave like riboflavin which shows a strong and persistent resonance at $\text{pH} < 1$, in agreement with the report of KUHN AND STRÖBELE[5] regarding the stability of this free radical. In addition to the aromatic quinones, we have observed the single electron oxidation-reduction intermediates of the conjugated compounds, triketohydrinene, and diacetyl to be stabilized in alkaline solution.

If the free radical life time is sufficiently short, the probability of a typical free radical reaction occurring remains remote, and it is interesting to observe that benzoquinone, for example, at pH 7.0 appears to pass back and forth from reduced to oxidized forms with no side reactions. At pH 8.0 there is already a very slight color change which evidences the existence of side reactions, and at pH 9.0 there appears the brown or blackish insoluble material which may be supposed to be a polymeric aromatic material similar to the melanin type pigments. It is in this pH range (8–9) and presumably for this reason that the quinhydrone electrode becomes unsatisfactory.

As the stability of the free radical increases, the likelihood of free radical chain reactions and polymerizations increases as well. One may suppose that the normal biological role of these compounds is insured *in vivo* through control of pH and the participation of antioxidants. Thus the mold pigments of the substituted anthraquinone type may be deterred from stable free radical formation by the supposedly acid environment provided by these organisms.

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