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OXYGEN ACTIVATION.

II. THE EFFECT OF CATECHOLAMINES*

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SUMMARY

1. Adrenal hormones greatly accelerated the autoxidation of *p*-hydroquinone by activating the O_2 molecule through complex formation by charge transfer.

2. O_2 activation by *o*-diphenolic compounds was also observed by using *p*-phenylenediamine as substrate. This explains the abnormally high O_2 uptake by the diamine in the presence of melanoma extracts, DOPA or catechol (the Greenstein–Riley effect).

3. The kinetics of catechol autoxidation is consistent with the view that one molecule is oxidized while a second catechol molecule acts as activator.

4. It is suggested that one of the primary roles of catecholamines is to activate O_2.

INTRODUCTION

The possible effect of complex formation by charge transfer in accelerating biochemically important reactions is under study in our laboratories (*cf.* refs. 1, 2; G. CILENTO AND I. POLACOW, unpublished observations). Theoretically, if towards a certain species a reactant is a better complex former when in the transition state than in the ground state, then this species will catalyze the reaction. A few examples of such a catalysis are known (*cf.* refs. 1–4; G. CILENTO AND I. POLACOW, unpublished observations).

O_2 shows a peculiar behaviour, as acceptance of one electron will favour entrance of a second electron[5]. Therefore in autoxidation reactions O_2 might be a better electron acceptor in the transition state than in the ground state whereby it is conceivable that these reactions may be accelerated by suitable electron donors. In this way we have explained[2] the catalytic effect of catechol in autoxidation reactions.

In view of the potential biological importance of this class of *o*-diphenolic compounds we have extended the investigation along the following lines: (i) assay of adrenal hormones; (ii) use of other substrates, namely *p*-phenylenediamines; and (iii) examination of the kinetics of catechol autoxidation.

EXPERIMENTAL

L-Epinephrine (Sigma Chemical Company) and norepinephrine (City Chemical Company) were used without further purification. Catechol and *p*-hydroquinone were

Abbreviation: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

* The work in ref. 2 constitutes the first paper of the series.

described in an earlier paper[2]. TMPD·2HCl was purchased from the British Drug Houses Ltd.; the free base was obtained by adding alkali to an aqueous solution, filtering and drying the product *in vacuo*. *p*-Phenylenediamine (Schering) was carefully sublimed. Organic solvents were purified by conventional procedures[6].

Buffers were prepared according to GOMORI[7], and the pH was determined with a Metrohm potentiometer. Whenever necessary, the pH of the mixture and of the controls were carefully equalized.

All solutions were prepared immediately before use.

Absorption spectra were taken with a Cary 14 recording spectrophotometer. To follow the catalyzed and uncatalyzed reactions, absorbances as a function of time were measured with a Beckman D.U. spectrophotometer; however, at the end of the experiment the spectrum was taken in order to be sure that it corresponded to that of the expected product.

Cells of 1 cm optical path were used throughout the work.

O_2 consumption was measured in air with a Warburg apparatus and the use of standard techniques.

RESULTS

Catalysis of p-hydroquinone oxidation by catecholamines

The first attempts to demonstrate spectrophotometrically that adrenal hormones speed up semiquinone formation from *p*-hydroquinone—as observed with catechol—were inconclusive because of secondary reactions[2]. Some success has now been achieved by using 0.1 M phosphate buffers (pH 7.2–7.9) mixed with methanol, acetone or dioxane. One of the best results is shown in Fig. 1; the main absorption developed by the complete system after the initial stage is that of noradrenochrome, whereas no such absorption appears in the hormone control. Increased adrenochrome formation can be detected even at low pH where no spontaneous oxidation of *p*-hydroquinone is observed (Fig. 2). Therefore adrenochrome formation must be due to increased oxidative processes occurring in the complete system.

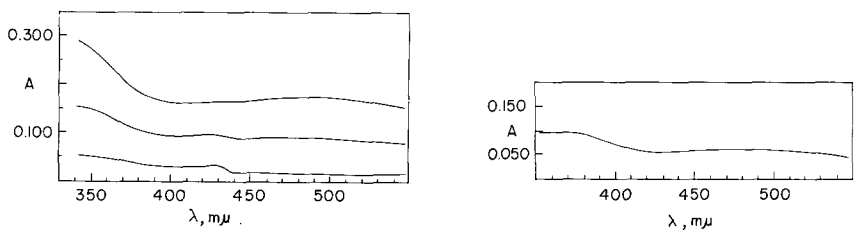

Fig. 1. The effect of 4.5 mM norepinephrine upon the autoxidation of 0.1 M *p*-hydroquinone in 0.1 M phosphate buffer (pH 7.2) mixed with methanol (1:1; v/v). The curves represent the spectrum of the mixture against that of *p*-hydroquinone after 60 sec (lower curve), 300 sec (middle) and 570 sec. The upper and middle curves have been displaced vertically by 0.1 and 0.05 in the absorbance value, respectively. The hormone alone did not generate any significant new absorption. The spectrum changes progressively from that of *p*-semiquinone to that of noradrenochrome.

Fig. 2. Adrenochrome formation in a system 0.3 M in *p*-hydroquinone and 10 mM in epinephrine; 0.1 M phosphate buffer (pH 5.75). The curve represents the spectrum of the mixture against that of the hormone after 4400 sec. Under the same conditions the autoxidation of *p*-hydroquinone is negligible.

That abnormally high O_2 uptake occurs in a system of *p*-hydroquinone and adrenal hormones could however be nicely demonstrated manometrically (Figs. 3 and 4). DOPA has not been assayed, but its activating effect upon the oxygen molecule has been observed in the *p*-phenylenediamine system as reported below.

As expected, resorcinol failed to substitute for *p*-hydroquinone.

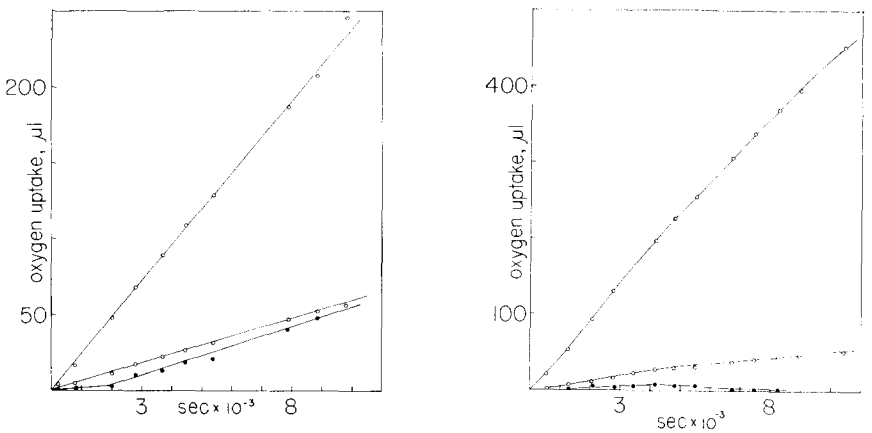

Fig. 3. Increased O_2 uptake in the system of 11 mM *p*-hydroquinone and 4.7 mM epinephrine; 0.1 M phosphate buffer (pH 7.50). Middle line, *p*-hydroquinone alone; lower, epinephrine alone.

Fig. 4. Increased O_2 uptake in a system of 11 mM *p*-hydroquinone and 20 mM norepinephrine; 0.1 M phosphate buffer (pH 7.50) mixed with methanol (1:1; v/v). Middle line, *p*-hydroquinone alone; lower, norepinephrine alone.

Oxidation of p-phenylenediamines catalyzed by o-diphenols

Since at neutral pH the semiquinone formed from the oxidation of *p*-phenylenediamines is much more stable than that from *p*-hydroquinone, especially in aqueous ethanol[8], we have investigated the effect of catechol upon the formation of the radical cation from *p*-phenylenediamine and from TMPD. The solvent was 0.01 M phosphate buffer (pH 6.4–6.9) mixed with methanol (1:1; v/v). Catalysis was clearly observed; a representative experiment is shown in Fig. 5. Conditions exist in which the rate of semiquinone formation either from *p*-phenylenediamine or

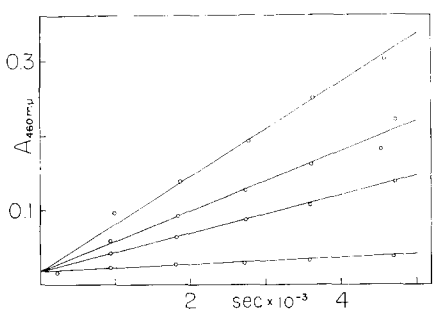

Fig. 5. The effect of catechol upon the autoxidation of 3.0 mM *p*-phenylenediamine as shown by the development of the semiquinone absorption maximum at 460 mµ; 0.01 M phosphate buffer (pH 6.4), mixed with methanol (1:1; v/v). Catechol concentration in mM (starting from the bottom), 0.0, 1.0, 2.5 and 5.1.

TMPD does not depend upon the catalyst concentration, a further indication that catechol does not act by way of its oxidation products[2].

Manometric data for systems of *p*-phenylenediamine with *o*-diphenols are available from studies of the Greenstein–Riley effect[9], that is the abnormally high O_2 consumption observed when melanoma extracts or homogenates are tested with *p*-phenylenediamine. RILEY[9] has found that the melanoma extracts can be substituted by DOPA and even by catechol, melanin-like products being formed. Our spectrophotometric investigation here too is in agreement with the manometric results.

Kinetics of catechol autoxidation

Since catechol acts as a catalyst by activating the oxygen molecule, it should also catalyze its own oxidation. The observed kinetics[10] are consistent with this view. Thus the rate of O_2 uptake obeys an expression of the type

$$\frac{B\,(C^-)^2\,(O_2)}{(C^-) + A}$$

where A and B are constants and (C^-) represents the concentration of the catechol monoanion. We have found that the above expression can be obtained from the steady-state treatment of the following mechanism

$$C^- + O_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} C^-.....O_2$$

$$C^-.....O_2 + C^- \xrightarrow{k_3} C^- + O_2^- + C^{\bullet}$$

in which one molecule of catechol is oxidized and the other acts as activator.

DISCUSSION

Two mechanisms appear possible for the synergism observed in a system of a catecholamine with *p*-hydroquinone or *p*-phenylenediamine as substrate: (i) the catecholamine catalyzes the oxidation of the substrate (to some extent the product formed may, in turn, oxidize the catecholamine); and (ii) the substrate catalyzes the oxidation of the catecholamine by activating the O_2 molecule.

In support of the first mechanism is the well established fact that catechol catalyzes the oxidation of *p*-hydroquinone[2], of *p*-phenylenediamines and of itself; moreover adrenal hormones themselves catalyze semiquinone formation from *p*-hydroquinone. Yet it is possible that the second mechanism also co-operates, since monoprotonated *p*-phenylenediamines can activate the O_2 molecule[11].

The manometric results confirm our interpretation[2] that the *o*-diphenol group activates the O_2 molecule without complete electron transfer, that is, without being cyclically involved by way of oxidation–reduction stages. The present results strengthen the view, suggested earlier[2], that one of the primary functions of *o*-diphenolic compounds is to activate O_2 and thus to promote electron transfer.

It may well be that conditions for O_2 activation by *o*-diphenols are more favourable *in vivo*. If the amino group of catecholamines is fixed to a receptor the formation of adrenochrome is minimized. Alternatively, formation of adrenochrome-like products may be followed by formation of 5,6-dihydroxyindoles[12], and these should be very strong O_2 activators. It is interesting that adrenochrome catalyzes the autoxi-

dation of epinephrine and of ascorbic acid[13]; an induction period in the autoxidation of epinephrine (Fig. 3) is noteworthy.

Our results give an explanation at the molecular level of the Greenstein–Riley phenomenon and related effects. Of these, the weaker toxicity of *p*-phenylenediamine to melanoma-bearing mice than to their non-tumour controls[9] is readily explained by the increased metabolic oxidation of the drug through the catalytic effect of the excess of DOPA-like compounds present in the tumour.

REFERENCES

1 G. CILENTO AND D. L. SANIOTO, *Arch. Biochem. Biophys.*, 110 (1965) 133.
2 G. CILENTO AND K. ZINNER, *Biochim. Biophys. Acta*, 120 (1966) 84.
3 A. K. COLTER, S. S. WANG, G. H. MEGERLE AND P. S. OSSIP, *J. Am. Chem. Soc.*, 86 (1964) 3106.
4 A. K. COLTER AND L. M. CLEMENS, *J. Am. Chem. Soc.*, 87 (1965) 847.
5 P. GEORGE, *International Symposium on Oxidases and Related Oxidation–Reduction Systems, Amherst, Mass.*, Vol. 1, Wiley, New York, 1964, p. 3.
6 A. I. VOGEL, *A Text-book of Practical Organic Chemistry*, Longmans, Green and Co., London, 1964.
7 G. GOMORI, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Academic Press, New York, 1955, Vol. I, p. 138.
8 L. MICHAELIS, M. P. SCHUBERT AND S. GRANICK, *J. Am. Chem. Soc.*, 61 (1939) 1981.
9 V. RILEY, *Proc. Soc. Exptl. Biol. Med.*, 98 (1958) 57.
10 M. A. JOSLYN AND G. E. K. BRANCH, *J. Am. Chem. Soc.*, 57 (1935) 1779.
11 G. CILENTO AND K. ZINNER, *Biochim. Biophys. Acta*, 143 (1967) 93.
12 R. A. HEACOCK, *Chem. Rev.*, 59 (1959) 181.
13 J. E. FALK, *Biochem. J.*, 44 (1949) 369.