Kinetics of Degradation in Solution of Epinephrine by Molecular Oxygen

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The rate of degradation of epinephrine in aqueous solution at pH 5 by molecular oxygen has been shown to be extremely complex functions of both the amine and oxygen concentrations. The kinetic studies, based on chromatographic isolation of epinephrine as its triacetate, indicate that at the pH used (*a*) the degradation products had no detectable effect on the observed oxidative rate, (*b*) the dependency on the catecholamine ranged from 1.0 to 0.5 depending on oxygen tension, and (*c*) conversely, the dependency on oxygen varied from over 1 to less than 0.4 depending on epinephrine concentration.

THE MECHANISMS and kinetics of the reaction between molecular oxygen and epinephrine have presented a challenge to pharmaceutical workers ever since the isolation of the hormone in 1901 by Takamine. Despite the long history of effort made in these directions, however, only a few reliable basic landmarks have been established concerning the reaction system because of (*a*) the lack of a suitable analytical procedure for the intact catecholamine and (*b*) the complexity of the oxidative process involved. Such paramountly important information as the rate dependencies of the concentration of the amine and oxygen had not been previously determined. In the present report the results of an investigation into these basic relations are given.

The influence of various factors on the rate of loss of epinephrine from its solutions has been attempted previously by numerous workers using a number of different assay techniques. Berry and West (1) have investigated the stability of epinephrine at different hydrogen ion concentrations by using a bioassay based on the response of a frog's heart to the drug. Notable among those methods based on colorimetric determinations was that of Mazur, Green, and Shorr (2) who studied the effect of drug concentration. Probably the most widely used method has been one based on some oxygen uptake technique. Wang (3), Trautner and Bradley (4), and Chaix, Chauvet, and Jezequel (5) have offered contributions based on this method of study. In none of the earlier investigations, unfortunately, was the rate of disappearance of the catecholamine itself observed. Since the oxygen uptake technique measures gas consumption, not only by epinephrine but also by a number of its breakdown products which are even stronger reducing agents, results obtained cannot be directly related to the primary reaction. Bioassay methods when used to follow epinephrine concentration also suffer from the fact that oxidation products can interfere with the determination. Ruiz-Gijon (6) has pointed out that adrenalinequinone, an oxidation product of epinephrine, has the same physiological properties as epinephrine. The results of any method based on a colorimetric determination should properly apply only to the catechol chromophore or the presence of phenolic hydroxyl groups.

The present investigation offers, for the first time, experimental results concerning the rate dependencies on the concentration of epinephrine and oxygen, based on a method which follows the loss of the drug by a direct and reproducible analysis of the intact molecule. The procedure was based on a modification of the U.S.P. XV method which successfully determines epinephrine in the presence of its oxidation products (7). The advantages that such a method for determining drug concentration holds over those techniques used by earlier workers is obvious.

EXPERIMENTAL

The dependency of the rate of autoxidation of epinephrine with respect to drug and oxygen concentrations was studied by reacting solutions of the amine at pH 5.0, selected for a convenient rate of degradation, and an ionic strength of 0.5 μ under various oxygen pressures at 65°.

Solutions of epinephrine at concentrations between 5×10^{-3} M and 0.2 M were prepared by weighing amounts of epinephrine base,[1] reported by the manufacturer and found on analysis to be more than 98% pure, into 0.05 M acetate buffer. The pH was adjusted with acetic acid and the ionic strength brought to the desired value by adding calculated amounts of potassium chloride. All

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[1] C. H. Boehringer Sohn, West Germany, m.p. 215°, specific rotation −53°.

hydrogen ion concentrations were measured at room temperature on the Beckman model H pH meter using a glass indicator electrode with a reference calomel electrode.

A reaction vessel containing the solutions of epinephrine was so designed as to permit equilibration with oxygen at a particular partial pressure, withdrawal of samples without disturbing that atmosphere, stirring of the solution, and storage in a constant temperature bath at 65.0 ± 0.2°. Figure 1 shows this apparatus. After the introduction of the solution into the vessel the atmosphere above the liquid was evacuated and the entire apparatus allowed to come to temperature equilibrium. The reaction was considered initiated with exposure to the oxygen atmosphere.

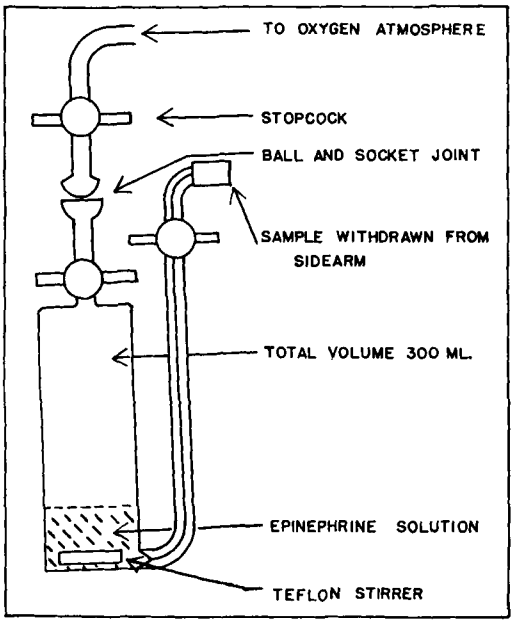


Fig. 1.—Schematic representation of the reaction vessel used in studying the rate of degradation of epinephrine at various drug concentrations and oxygen pressures.

A 5-gallon jar served as the oxygen source and was attached to the apparatus by glass tubing. By displacing known volumes of water with high purity oxygen and nitrogen, any desired partial pressure of oxygen could be obtained. A mercury manometer was attached to the system and the oxygen-nitrogen mixtures were maintained at a positive pressure of 3 to 4 cm. of mercury. This manometer served as a check to insure the maintenance of a constant oxygen pressure throughout any particular determination. The amount of oxygen added was such that if all of the epinephrine present were to degrade, there would be no more than a 15% decrease in the pressure above the solution. The error represented a maximum that was never realized since the epinephrine never completely degraded nor did the manometer indicate such a drastic change in pressure. For those studies above one atmosphere, the reaction vessel was attached directly to the oxygen tank through a trap and the gauge reading taken as the pressure above the solution.

After suitable lengths of time, samples of the desired volume were withdrawn quantitatively from the sidearm of the vessel into a pipet. The solution was then transferred to a beaker and frozen until the time of assay by the modified U.S.P. XV procedure (7). The amount of solution withdrawn depended on the concentration of the epinephrine and attempts were made so that the acylated solution initially contained the equivalent of 10 mg. of epinephrine prior to its transfer to a chromatographic column. For example, in the analysis of the 0.2 *M* epinephrine solution, 3 ml. of the solution was withdrawn, diluted to 25 ml. with distilled water, and 2 ml. of this solution acylated after adjusting the final volume to 5 ml. The acylated mixture was transferred to a column for chromatographic separation after mixing with 5.6 Gm. of Celite 545 as described in a previous study (7).

RESULTS

The dependence of the reaction with respect to the drug concentration was found to vary with the oxygen tension present and ranged from a simple proportionality at low oxygen pressures to a half-order at higher oxygen concentrations. When the oxygen tension was held at 0.13 atmospheres and the concentration of epinephrine followed as a function of time, plots represented by Fig. 2 were obtained. It should be noted that in this particular determination and in each of the other kinetic studies made, the degradation proceeded with no apparent lag phase. Reactions which follow a free radical path are often characterized by an initial lag time during which period intermediates accumulate.

Since it was not possible to determine clearly the dependency of the reaction with respect to the substrate by merely plotting various functions of the concentration against the time, the changes in the initial rate with initial concentrations of the drug

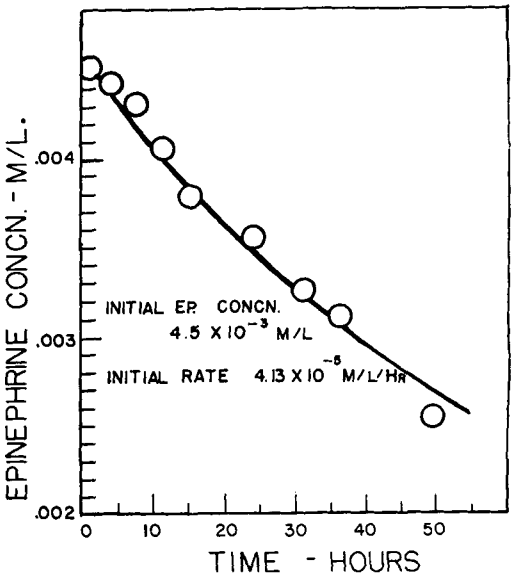


Fig. 2.—Epinephrine concentration (moles/L.) as a function of the time (hr.) at 0.13 atmosphere oxygen, pH 5, ionic strength 0.5 μ, and temperature 65° where the initial epinephrine concentration was 4.5 $\times 10^{-3}$ *M*.

TABLE I.—EFFECT OF EPINEPHRINE CONCENTRATION ON THE INITIAL RATE OF DRUG DEGRADATION AT VARIOUS OXYGEN PRESSURES

Oxygen Pressure, Atmospheres											
0.13		0.26		0.53		1.1		2.0		2.6	
A[a] × 10^3	B[a] × 10^5	A × 10^3	B × 10^4	A × 10^3	B × 10^4	A × 10^3	B × 10^4	A × 10^3	B × 10^4	A × 10^3	B × 10^3
4.5	4.15	7.00	1.70	4.8	2.7	4.10	9.5	4.44	7.5	4.90	1.51
5.03	6.20	50	10.7	9.6	3.76	9.70	11.8	4.78	10.4	9.40	2.40
10.3	11.4	21.3	4.60	46.4	13.2	50.5	19.8	9.93	18.7	49.0	5.90
11.0	10.8	170	29.3	183	33.4	51.8	21.0	16.6	28.8	192	7.30
43.6	67.5	...	...	...	...	45.8	21.3	47.5	43.3	...	...
...	...	...	...	...	...	354	75.0	186	95.0	...	...
...	...	...	...	...	...	...	...	188	67.5	...	...

[a] A = Initial epinephrine concentration (moles/L.); B = initial rate (moles/L./hr.)

TABLE II —FIRST-ORDER RATE CONSTANTS IN THE DEGRADATION OF EPINEPHRINE AT 0.13 ATM. OXYGEN

Initial Epinephrine Concn (moles/L × 10^3)	Graphically Obtained Rate Constant × 10^2 (hr.$^{-1}$)
4.51	1.1
5.03	0.9
10.3	1.0
11.0	1.0
43.6	1.5
183	1.1

were determined. The approach largely obviates participation of various degradation products in the epinephrine reaction *per se*, since these are assumed to be quite low in concentration in the beginning.

The initial rates were found by taking initial slopes of lines as shown in Fig. 2 expressed as moles/L./hr. By varying the initial concentration of the drug, the change in the initial rate as a function of drug concentration could be determined. The results of these determinations at 0.13 atmospheres oxygen appear in the first column of Table I. To determine the dependency of the rate with respect to the epinephrine concentration at this oxygen tension the data appearing in Table II were plotted on a log-log scale. It is evident in this type of plot that the slope of the line is identical with the dependency of the rate with respect to drug concentration.

By choosing six different oxygen pressures above the solutions and applying the same principles it was possible to determine the effect that oxygen had on the dependency with respect to epinephrine. These data are also summarized in the columns of Table I. The log-log plots establishing the dependency of epinephrine are given in Fig. 3. The manner in which the drug dependency is affected by the oxygen pressure is represented in Fig. 4. In this plot the order of the reaction with respect to the epinephrine concentration is shown as a function of the oxygen pressure and it is seen that the order changes quite rapidly initially as the oxygen pressure increases. At a value of about one atmosphere the order tended to level off and remained steady at about a half-order dependence.

At lower oxygen pressure the order with respect to the substrate is apparently first order. If the data of Fig. 2 were plotted on a semilogarithmic scale a straight line should be obtained, provided that degradation products did not alter the rate of reaction.

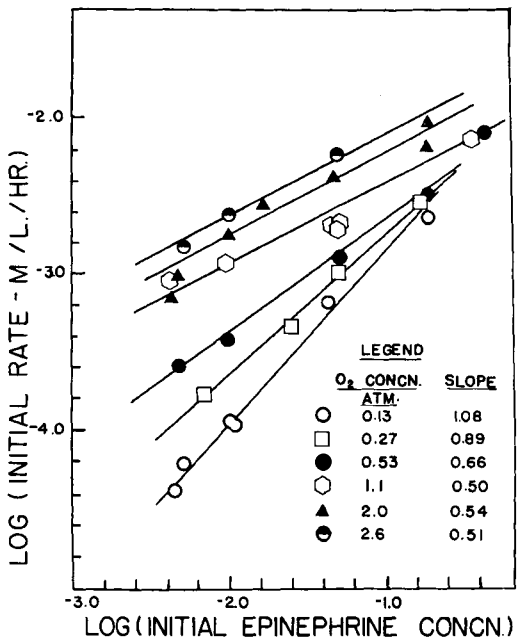


Fig. 3.—The logarithm of the initial rate of epinephrine degradation as a function of the logarithm of the initial epinephrine concentration at different oxygen pressures.

The logarithmic plot is presented in Fig. 5, and it is evident that the first-order dependency of the drug holds over a considerable loss of the substrate. The first-order rate constants calculated from plots as Fig. 5 should be independent of the initial epinephrine concentrations. Table II shows these rate constants obtained for different runs made at several initial concentrations of the drug. The variation observed here was only about 10% and well within the deviation to be expected.

The rate dependency of the catecholamine oxidation on oxygen tension above the solution was determined from data already presented in the preceding section. Since the initial rates of the loss of epinephrine having exactly the same initial epinephrine concentration were not determined under different oxygen tensions it was necessary to obtain these points by interpolation of such data as has been obtained. Each initial rate at a particular oxygen pressure and initial epinephrine concentration was interpolated from initial rates obtained for given different initial epinephrine concentrations

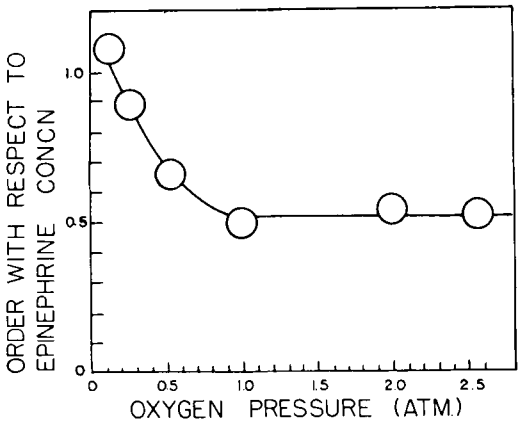


Fig. 4.—The order of the rate of epinephrine autoxidation with respect to drug concentration as a function of oxygen pressure (atmosphere).

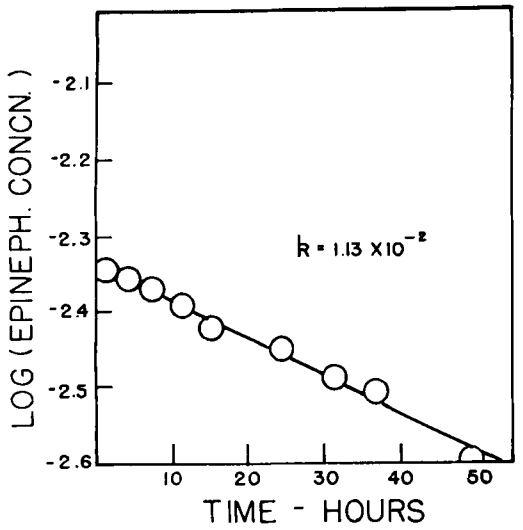


Fig. 5.—The logarithm of the epinephrine concentration as a function of the time (hr.) at 0.13 atmosphere oxygen, pH 5, ionic strength 0.5 μ, and a temperature of 65° where the initial epinephrine concentration was 4.5×10^{-3} M.

making use of Fig. 3. In this way the rates at six different oxygen pressures all at the same initial epinephrine concentration were found. Data were thus obtained for seven different initial epinephrine concentrations ranging from 0.0032 to 0.2 M. The interpolated data are shown plotted logarithmically in Fig. 6 as the initial rate against the oxygen tension. The slope of each line corresponds to the dependence of the autoxidative rate with respect to oxygen concentration at various initial epinephrine concentrations. It is apparent from Fig. 6 that as the concentration of epinephrine is increased, the slope or order with respect to oxygen concentration decreases. The apparent order is strongly affected by the epinephrine concentration as shown in Fig. 7, decreasing gradually at the higher levels of the drug. A fractional dependency with respect to oxygen concentration in an oxidative process is apparently not uncommon.

The effects of solutions of epinephrine initially at

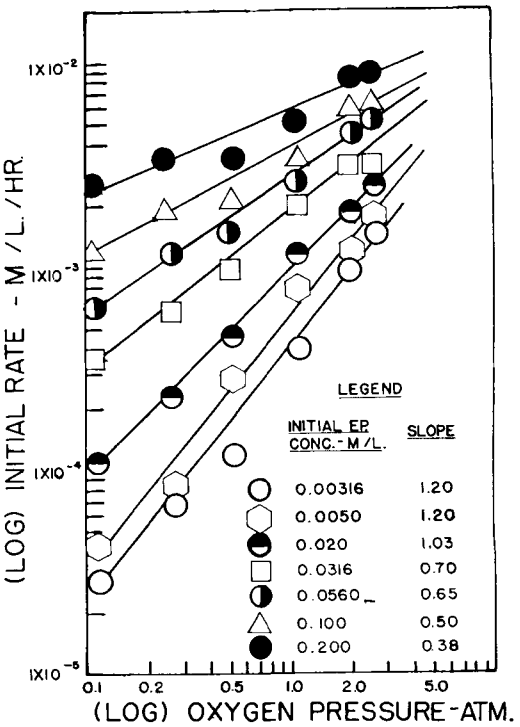


Fig. 6.—The logarithm of the initial rate of epinephrine degradation as a function of the logarithm of the oxygen pressure at different initial concentrations of epinephrine.

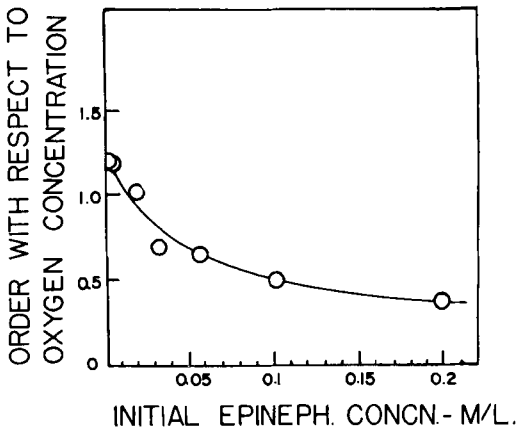


Fig. 7.—The order of the rate of autoxidation of epinephrine with respect to oxygen concentration as a function of the epinephrine concentration.

0.01 M and an ionic strength of 0.5 containing concentrations of 6.8×10^{-3} M potassium chloride, 2.2×10^{-4} M copper (II) acetate, and no copper or cyanide were compared. Oxidation appeared to take place even in the absence of copper although there can be no question that copper acted as an effective catalyst for epinephrine in aqueous solutions under the influence of oxygen. The graphs showing the dependence of the epinephrine concentration as a function of time did not exhibit a lag phase and thus were similar to those obtained in the epinephrine dependence study. The data

TABLE III.—EFFECT OF POTASSIUM CYANIDE AND COPPER (II) ACETATE ON THE DEGRADATION OF EPINEPHRINE

	Concentration of Additive, moles/L.	Half-Order Rate Constant $\times 10^2$, $(\text{moles/L.})^{1/2}$hr.
KCN	6.8×10^{-4}	1.0
KCN	6.8×10^{-3}	1.0
KCN	3.4×10^{-2}	1.2
KCN	6.8×10^{-2}	1.1
$Cu(OAc)_2$	2.2×10^{-4}	3.6
	0.00	0.8

yielded linear half-order plots. The resulting rate constants for each of the three solutions, potassium cyanide, copper acetate, and no copper or cyanide, are shown in Table III. It can be seen that the copper-containing solution degraded almost four times faster than the one containing potassium cyanide, and five times that of the one containing no copper or cyanide.

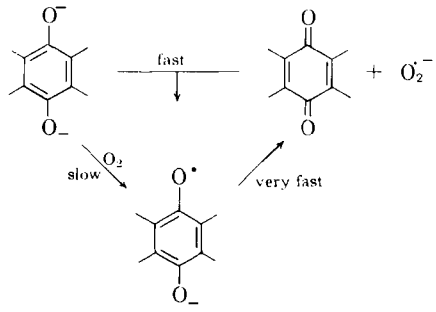
The rate of degradation of the solution containing the cyanide salt was more rapid than that containing none. This fact prompted a study of the effect of varying potassium cyanide concentrations investigating some possible catalytic action. The data, also shown in Table III, indicates, however, that even though the concentration of potassium cyanide was increased a hundredfold, no increase in rate constant was noticed.

DISCUSSION

The results of the studies on the kinetics of autoxidation of epinephrine as presented in the previous section are essentially summarized in Table IV. Orders referred to in the table were obtained from the dependencies of the initial rates of disappearance of the amine from solution.

At low concentrations of both reactants, the degradative reaction seems to be effectively second order. This simple dependence of the rate of reaction is not surprising but does not necessarily imply that the underlying mechanism is simple. Although Joslyn and Branch (8) reported that the oxidation of catechol proceeded with a first-order dependency on catechol concentration, this direct proportionality was apparently the consequence of a highly complex mechanism. Close analogies of the catechol degradation to the epinephrine oxidation cannot be made since in the case of epinephrine the intermediate species apparently involve the nitrogen. According to Wang (3) the rate of oxygen uptake is markedly reduced if a methyl radical replaces the primary nitrogen on epinephrine.

If the concentration of epinephrine and oxygen are both high, the rate of drug oxidation depends on the oxygen concentration raised to a fractional power and varies directly with the square root of the epinephrine concentration. The appearance of fractional orders strongly suggests that the degradative mechanism of epinephrine oxidation may be free radical mediated. This behavior is similar in some ways to the autoxidation of durohydroquinone, 2,3,5,6-tetramethyl hydroquinone, studied by James and Weissberger (9). According to their postulations the following scheme is indicated for this system



Such a scheme would rationalize the one-half power dependency of epinephrine at higher concentrations but, in turn, requires some sort of lag phase for the buildup of the quinoid species and a catalytic activity on the part of the oxidation products of the catecholamine. The present study gives neither any evidence of a lag phase nor data which suggests that the reaction is autocatalytic. The reaction seems to be totally unaffected by the accumulation of products during its course. This observation does not, of course, preclude the possibility that some highly unstable but dimerizable intermediate may play a catalytic role.

It should be pointed out that another work based on oxygen uptake by Trautner and Bradley (4) obtained evidence for major catalytic effects on the part of adrenochrome. The present results do not suggest that intermediate species cannot act catalytically but that these do not accumulate significantly in these solutions. It is interesting to note the part that the hydrogen ion concentration may contribute to the accumulation of intermediates. From experimental evidence it has been shown that an equilibrium exists between hydroquinone, quinone, and semiquinone according to the following scheme

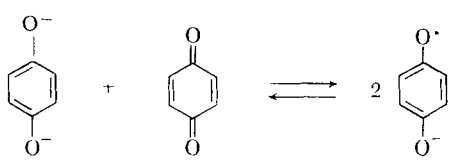


TABLE IV.—DEPENDENCE ON OXYGEN AND EPINEPHRINE CONCENTRATION OF THE INITIAL RATE OF EPINEPHRINE DISAPPEARANCE FROM SOLUTION AT HIGH AND LOW EPINEPHRINE AND OXYGEN CONCENTRATIONS

	Case	Order with Respect to Reactant: Oxygen	Order with Respect to Reactant: Epinephrine
I	Low epinephrine and low oxygen concentration	1^+	1.0
II	High epinephrine and high oxygen concentrations	Less than 0.5	0.5
III	High epinephrine and low oxygen concentration	Less than 0.5	1.0
IV	Low epinephrine and high oxygen concentration	1^+	0.5

The equilibrium favors a semiquinone formation when the divalent hydroquinone is the reactive species present, but in an acid solution the semiquinone is unstable and the reaction shifts considerably to the left. On the basis of these results, the studies of Trautner at pH 6–8 would apparently favor a stable semiquinone intermediate in contrast to the present studies made at pH 5 where the radical may possibly be more reactive and in lower concentration.

The results of Wang would tend to support the postulated rapid formation of radicals and may explain the apparent absence of a lag time. Her studies (3) show that oxygen was consumed in relatively short periods of time in comparison to the loss of epinephrine in the present investigation. At pH 4.9, 50°, and an epinephrine concentration of 0.001 *M*, Wang reports an oxygen consumption of 22.8 μl. after 0.6 hours, where a total of 224 was consumed after 9 hours.

It is possible to compare the results of the present investigation based on a chemical assay for the intact drug with that of Wang. By relating the rate of oxygen consumption to the rate of disappearance of epinephrine, Wang showed no lag phase present at pH 4.9 in a temperature range of 38 to 50°. However, at higher pH values a lag time was experienced. By interpolation of Wang's data the rate at which oxygen was consumed at 65° under air, where the initial epinephrine solution was 0.001 *M* at pH 4.9, was 9.5×10^{-6} moles/L./hr. By interpolating the data of the present study at approximately the same conditions, 0.001 *M* of epinephrine initially at pH 5 under 0.27 atm. oxygen, the rate was found to be 3.2×10^{-5} moles/L./hr. A close but not exact agreement suggests that perhaps the rate at which oxygen is consumed is some multiple of the rate of disappearance of the drug.

Under those conditions of high epinephrine concentration and low oxygen tension, the rate varies with a fractional-order dependency of oxygen and a first-order dependence with respect to epinephrine concentration. In contrast to this behavior we have the fourth case in which oxygen dependency is greater than unity and epinephrine is of a fractional order at low epinephrine and high oxygen pressure. Undoubtedly both oxygen and epinephrine are important reactants in the oxidation although their exact roles are not known. It is especially difficult to postulate the role of oxygen since indications are that, at low epinephrine concentrations, the oxygen dependence is greater than unity. There have appeared in the literature reactions which depend on a three-halves order with respect to oxygen but these were generally reactions in which metals were present as catalysts. In the present study oxygen dependence is shown only as a function of epinephrine concentration.

Chaix, Chauvet, and Jezequel (5) have said that in the absence of heavy metals an oxidation of epinephrine will not take place. The present studies have shown that although copper catalyzes the autoxidative degradation, the reaction probably takes place in their apparent absence. When the effect of various potassium cyanide concentrations was investigated, a small increase in rate over those solutions with no cyanide or copper was noticed, rather than the expected decrease. The cyanide has been known to act as a strong complexing agent for heavy metals and it was expected that it would act as a scavenger for the metals present in our system. The metals thus removed should have been unavailable for catalytic action. The small increase in rate is perplexing but demonstrates that heavy metals may not be needed for autoxidation.

Another evidence which suggests that trace amounts of metals in systems of the present study were not responsible for the degradation of epinephrine is based on the reproducibility that could be obtained. From Chaix's data an increase of copper ion concentration from 0 to 10^{-6} *M* or from 10^{-6} to 10^{-5} *M* would increase the rate of oxidation by a factor of two. Such increases in systems where the heavy metal concentration was not closely controlled would markedly affect reproducibility. The origin of free radicals may possibly come from oxygen and the organic species present and need not be initiated by heavy metals.

CONCLUSION

We are not prepared on the basis of the present work to present a complete mechanism sequence by which the observed results can be explained. The results suggest, however, that the overall reaction is an extremely complex one and, without question, involves free radical mediated sequences. The study appears to put to rest a number of previously unsupported concepts concerning the mode of oxidation of epinephrine at hydrogen ion concentrations approaching those commonly employed.

The behavior of this system as reflected in this study strongly points to formation of nonpersistent, dimerizable odd electron catalytic species. These probably act as the initial oxygen transfer agents. The results suggest, however, that none of the gross oxidation products possesses any significant catalytic effect.

From a purely practical and pharmaceutical standpoint it is evident from the data presented that for systems exposed to high oxygen tension the rate of degradation is always less than first order and that the percentage of the drug lost per unit time is always less per unit time at higher concentrations than at lower concentrations. These and other conclusions possible from these results may be of value in formulation of the drug.

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