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The Influence of Catechol on the Stability of o-Benzoquinone in Aqueous Solutions

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Most of the phenolic oxidases which have been studied so far oxidize catechol, and hence the latter has found wide application as a substrate in the study of this class of enzymes. In the aerobic oxidation of catechol by phenolases several investigators,[1-3] have reached the conclusion that *o*-benzoquinone is the initial oxidation product. The enzymatic oxidation reaction, however, does not stop at the quinone stage, but continues with the consumption of an additional atom of oxygen per mole of catechol, yielding in its final stage highly colored solutions containing products of unknown chemical constitution.[4,5] Furthermore, *o*-benzoquinone is exceedingly unstable in aqueous solutions,[6-9] and experiences in these Laboratories have suggested that unoxidized catechol greatly influences this instability. In all probability the enzymatic oxidation of catechol, especially after the formation of some *o*-benzoquinone, must be quite complex, involving a series of consecutive as well as concurrent reactions. Therefore, any attempt to gain additional light on this complex enzymatic reaction demands that more information be obtained, not only concerning the disappearance of the *o*-benzoquinone in aqueous solutions but also concerning the influence exerted by the presence of unoxidized catechol on the disappearance of the *o*-benzoquinone. The study reported in this communication deals with the influence of catechol on the rate of disappearance of *o*-benzoquinone in aqueous solutions of varying initial concentrations of the latter and varying hydrogen ion concentrations.

The results obtained by Ball and Chen[10] in their study of the oxidation of catechol by ceric sulfate seem to justify these investigators' claim that in dilute solutions the oxidation of catechol to *o*-benzoquinone by this reagent is complete and practically instantaneous. Thus it is possible, by mixing varying excessive amounts of catechol with known amounts of ceric sulfate, to prepare aqueous solutions of known initial concentrations of *o*-benzoquinone and unoxidized catechol. If conditions are chosen such that the rates of disappearance of the *o*-benzoquinone are not too great, *i. e.*, dilute solutions and suitable hydrogen ion concentrations, then the effect of the unoxidized catechol on the rate of disappearance of the *o*-benzoquinone can be followed by the usual methods of quinone determination. In this study iodimetric titration was employed, and the procedure followed is given in the legend of Fig. 1.

Instability of *o*-Benzoquinone.—The results obtained in solutions buffered to *p*H values of approximately 4.0 are shown graphically in Fig. 1, and the rest of the data which were obtained from identical experiments carried out in solutions buffered to *p*H values of approximately 5.0 and 5.5 have been compiled along with the *p*H 4.0 data in Table I. The actual curves in this one case (*p*H 4.0) have been used to point out more strikingly the effect of the unoxidized catechol on the rate of disappearance of the *o*-benzoquinone. In Fig. 1, the number of cc. of the standard thiosulfate solution used to titrate the iodine liberated by the *o*-benzoquinone have been plotted against time, and the two sets of curves, obtained in this manner, indicate the rates of disappearance of the *o*-benzoquinone in the presence of different amounts of unoxidized catechol for two different initial concentrations of the *o*-benzoquinone (see Table). The curves 1 and 1′ in this figure and the corresponding data for *p*H 5.0 and 5.5 in the table indicate the rates of disappearance of the *o*-benzoquinone in water containing no catechol. Thus by a comparison of the values for the initial slopes (s and s')[12] of these curves 1 and 1′ it will be noted that the initial slope or rate of disappearance of the *o*-benzo-

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(12) Since the majority of the reaction curves failed to follow any simple reaction order, the initial slopes of the curves have been used for the sake of comparison of the relative rates of disappearance of the *o*-benzoquinone.

TABLE I

Dilute Solution; 10 mg. *o*-Benzoquinone per 250 cc.; $Ce(SO_4)_2$ = 0.00073 *N* = 60.5 mg. in 250 cc. Used 1% KI solution. 60.5 mg. $Ce(SO_4)_2 \equiv$ 10.02 mg. $C_3H_4(OH)_2 \equiv$ 10.00 mg. $C_3H_4O_2 \equiv$ 1.88 cc. of 0.00971 *N* $Na_2S_2O_3$.

Curve	Mg. catechol added 0 time	Mg. excess catechol	% oxid.	*p*H 4.0 (app.) Fig. 1 *p*H	Initial slope, *s*	Ratio slopes	*p*H 5.0 (app.) *p*H	Initial slope, *s*	Ratio slopes	*p*H 5.5 (app.) *p*H	Initial slope, *s*	Ratio slopes
1	10.02	0	100	4.06 → 4.09	0.0300		5.00 → 5.03	0.0400		5.48 → 5.54	0.0467	
2	11.15	1.13	89.6	4.05 → 4.09	.0317	1.06	5.02 → 5.06	.0450	1.12	5.45 → 5.50	.0533	1.14
3	22.30	12.28	44.8	4.06 → 4.10	.0400	1.33	5.03 → 5.06	.0700	1.75	5.46 → 5.53	.0916	1.96
4	44.60	34.58	22.4	4.05 → 4.09	.0533	1.78	5.01 → 5.04	.1120	2.80	5.48 → 5.54	.1660	3.34
5	0			4.05	0							

More Concentrated Solution; 30 mg. *o*-Benzoquinone per 250 cc.; $Ce(SO_4)_2$ = 0.00219 *N* = 181.5 mg. $Ce(SO_4)_2$ in 250 cc.; used 3% KI soln.; 181.5 mg. $Ce(SO_4)_2 \equiv$ 30.06 mg. $C_6H_4(OH)_2 \equiv$ 30.00 mg. $C_3H_4O_2 \equiv$ 5.64 cc. of 0.00971 *N* $Na_2S_2O_3$.

Curve	Mg. catechol added 0 time	Mg. excess catechol	% oxid.	*p*H 4.0 (app.) Fig. 1 *p*H	Initial slope, *s'*	Ratio slopes	*p*H 5.0 (app.) *p*H	Initial slope, *s'*	Ratio slopes	*p*H 5.5 (app.) *p*H	Initial slope, *s'*	Ratio slopes
1′	30.06	0	100	3.95 → 4.03	0.117		4.90 → 4.97	0.178		5.30 → 5.44	0.215	
2′	35.50	5.44	84.9	3.92 → 3.99	.132	1.13	4.88 → 4.94	.220	1.23	5.31 → 5.50	.298	1.29
3′	71.10	41.04	42.4	3.92 → 4.01	.245	2.09	4.89 → 4.95	.513	2.88	5.31 → 5.47	.787	3.66
4′	142.0	111.94	21.2	3.93 → 4.02	.433	3.70	4.90 → 4.96	1.041	5.88	5.31 → 5.46	1.624	7.55
[a]5′	35.5	5.44	84.9	3.95 → 4.05	0							
[a]6′	142.0	111.94	21.2	3.95 → 4.06	0							

The data from the series of experiments run at *p*H values approximately 5.0 and 5.5, respectively, were plotted like the data obtained at *p*H approximately 4.0 and represented by Fig. 1, and the respective initial slopes were determined. For the sake of brevity, however, the curves have been omitted, but the values for the initial slopes are given in the table under the correct *p*H heading and opposite the corresponding curve number. The columns headed "Ratio-slopes," therefore, indicate the ratio of the slope of curve 2 to that of curve 1 or the slope of curve 3 or 4 to that of curve 1, etc.

[a] Hydroquinone instead of catechol.

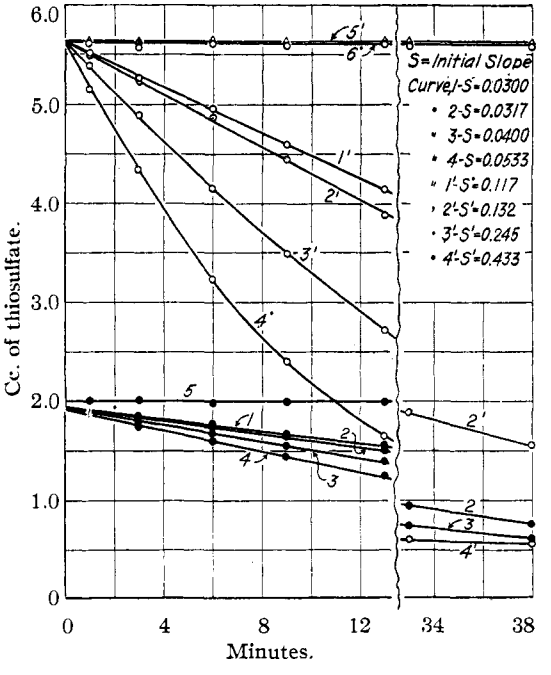

Fig. 1.—*Rate of disappearance of o-benzoquinone in the presence of different amounts of catechol at pH 4.0.*

Aqueous solutions of known initial concentrations of *o*-benzoquinone and unoxidized catechol were prepared in the following manner. Nitrogen gas was passed through dilute solutions of ceric sulfate (270 cc.), buffered with 0.1 *M* acetate buffer, contained in a 600-cc. reaction bottle provided with a three-hole stopper. (One hole for a pipet to withdraw samples, one for permitting the insertion of a glass tube so that the contents of the flask could be stirred with nitrogen, and the third serving as an outlet for the nitrogen.) After the reaction solution had attained the temperature of the thermostat (25 ± 0.02°) and most of the air had been swept out by the nitrogen, a 25-cc. sample of the solution was removed for *p*H determination. (Because of the great speed of oxidation of catechol by ceric sulfate, it was not possible to measure the *p*H of the initial reaction mixture. Therefore the *p*H of the buffered ceric sulfate solution was determined before the addition of the catechol and after the reaction had taken place. It will be observed in the data given in Table I that the difference between the two *p*H values is very small except in the more concentrated solutions at hydrogen-ion concentrations where the acetate loses considerable of its effectiveness as a buffer.) The latter operation was then followed by the addition of 5 cc. of freshly prepared aqueous solution of given amounts of catechol at zero time (reaction volume 250 cc.). Within the first fifteen minutes several 25-cc. samples were withdrawn at various time intervals and added immediately to molar sulfuric acid. This operation was immediately followed by the addition of 10 cc. of potassium iodide solution of the proper strength. (Cerous sulfate catalyzes the aerobic oxidation of hydriodic acid[11] and therefore unless careful precautions are taken to exclude all air from the system, the titration of ceric sulfate solutions by the iodine–thiosulfate method leads to too high values of iodine. This error can be greatly minimized however by passing nitrogen through the system and using only a small excess of hydriodic acid. Curve (5) in Fig. 1 is about 6% too high because of this reason. This effect of cerous sulfate completely disappears however in the presence of catechol.) The samples were then placed in the dark for fifteen minutes before titrating with standard thiosulfate solution the iodine set free by the *o*-benzoquinone.

The ceric sulfate solutions were prepared with ceric ammonium sulfate $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$ of analytical grade of purity. The catechol used was the chemically pure Eastman Kodak Co. product of sharp melting point. As pointed out by Ball and Chen[10] the use of ceric sulfate as an oxidizing agent is restricted to acid solutions. It can be used as an oxidizing agent at these *p*H values only in acetate buffers. The usual hydrolysis of ceric sulfate at these *p*H values is retarded by the presence of the acetate ions; its oxidizing ability remaining unaltered.

quinone increases with increase in *p*H, and increases with increase in initial concentration of the *o*-benzoquinone.

Fieser and Peters[13] in their study of the disappearance of β-naphthoquinone in weakly acid solutions reached the conclusion that the reaction was of the first order, and that the first and slowest step in the reaction was simply a hydration of the quinone. Furthermore, they expressed the opinion that this first order reaction is characteristic of all unsubstituted *o*-quinones. Our data corresponding to the rates of disappearance of the *o*-benzoquinone in the more dilute solutions with no unoxidized catechol present agree quite well with the claim of Fieser and Peters, *i. e.*, these rates follow fairly closely the first order reaction requirements. However, in the more concentrated solutions there is a considerable variation from these requirements and this variation increases with increase in *p*H of the system. The *o*-benzoquinone disappears too rapidly to be accounted for by a simple first order reaction. For the latter to hold, a three-fold increase in the initial concentration of the *o*-benzoquinone should bring about the same relative (three-fold) increase in the initial rate of disappearance of the *o*-benzoquinone. Using the initial slopes (s) and (s') of these curves 1 and 1′ as measures of the initial rates of disappearance of the *o*-benzoquinone, the ratios of these slopes (s'/s) is for each hydrogen ion concentration considerably and increasingly greater than 3. (See Table: *p*H = 4, s'/s = 3.9; *p*H = 5, s'/s = 4.4; *p*H = 5.5, s'/s = 4.6.)

Influence of Catechol on the Instability of the *o*-Benzoquinone.—As already stated in the introduction, the main object of this study was to investigate further the accelerating influence on the rate of disappearance of the *o*-benzoquinone, caused by the presence of unoxidized catechol. The initial slopes (s) for curves 1, 2, 3 and 4, as shown in Table I for three different *p*H values, all show a progressive increase in the rate of disappearance of the *o*-benzoquinone with increase in amount of unoxidized catechol present. In these experiments the initial concentration of *o*-benzoquinone was in each case 10 mg. per 250 cc. of reaction solution; this initial concentration of *o*-benzoquinone being obtained in the manner previously described in the legend of Fig. 1. On the other hand, experiments represented by the data from curves 1′, 2′, 3′ and 4′ as given in Table I were conducted in a manner exactly analogous to those just mentioned, except that the initial concentration of the *o*-benzoquinone was three times as great, *i. e.*, 30 mg. in 250 cc. of solution. Here again, a comparison of the initial slopes (s') shows that the initial rate of disappearance of *o*-benzoquinone is materially increased by the presence of unoxidized catechol. The interesting and important observation to make is that the effect of the unoxidized catechol on the initial rate of disappearance of the *o*-benzoquinone appears from the data available to increase directly with the increase in amount of unoxidized catechol present. This conclusion can be reached in two ways from the data given in Table I, *i. e.*, by visual comparison, or better still, graphical comparison, of the increase in the ratio of slopes (slope when excess catechol is present/slope at 100% oxidation) for any given *p*H with the increase in the amount of unoxidized catechol present, or by comparing the difference in the initial slopes (slope when excess catechol is present minus the slope at 100% oxidation) with the increase in the amount of unoxidized catechol present.

Instability of *o*-Benzoquinone and Hydrogen Ion Concentration.—As previously pointed out, the rate of disappearance of the *o*-benzoquinone in aqueous solutions containing no catechol increases with decrease in hydrogen ion concentration. It is interesting to note therefore by inspecting the change of the initial slopes (either s or s') with *p*H in the table, that the influence of the presence of the unoxidized catechol on the initial rate of disappearance of the *o*-benzoquinone also increases with *p*H of the system. For example, at *p*H 4.0 in the more dilute solution (10 mg. *o*-benzoquinone per 250 cc.) the initial rate of disappearance of the *o*-benzoquinone when the catechol is 22.4% oxidized (34.58 mg. of unoxidized catechol present) is 1.78 times as great as when no excess catechol is present, *i. e.*, 100% oxidation; while at *p*H 5.5 under the same experimental conditions the initial rate is increased by 3.34 times. It can be seen that in the more concentrated solutions the influence of the presence of unoxidized catechol on the rate of disappearance of the *o*-benzoquinone increases even more with increase in *p*H. Thus, at *p*H 4 the presence of 112 mg. of catechol (142.0 − 30.06) in the solution containing 30 mg. of *o*-benzoquinone increased the initial rate of disappearance of the quinone 3.7 times faster than when no catechol was present, while at *p*H 5.5 the same amount of

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catechol increased the initial rate 7.55 times. In other words, for the same ratio of catechol to the quinone in dilute and more concentrated solutions the increase in the accelerating influence of the catechol on the initial rate of the disappearance of the *o*-benzoquinone with increase in *p*H is noticeably greater in the more concentrated solutions (7.55/3.7 = 2.4 and 3.34/1.78 = 1.8).

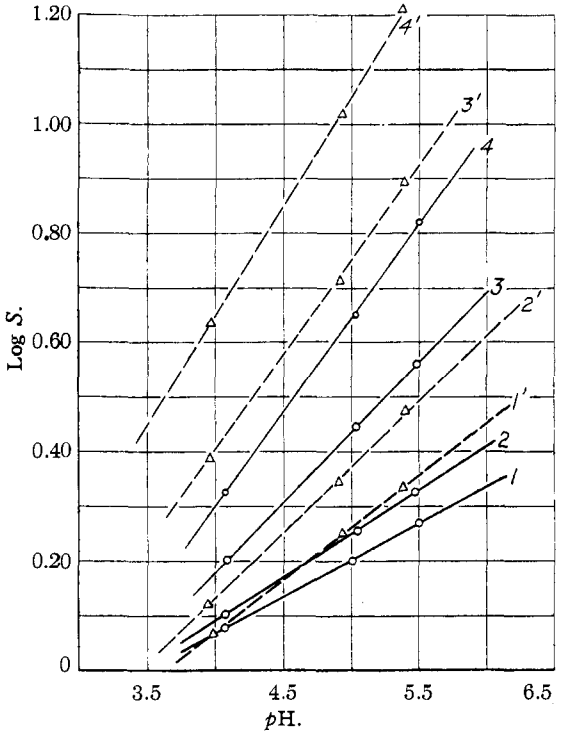

Fig. 2.—Curves indicating the linear relationship between the *p*H and the logarithm of the initial rate of disappearance of *o*-benzoquinone in the presence of different amounts of catechol. The initial slopes (*s* and *s'*) given in Table I have been used as measures of the initial rates of disappearance of the *o*-benzoquinone. The three points occurring on each curve in this figure correspond to the respective values of the initial slopes listed in the table opposite the same curve number. In order to plot positive values of the logs of these initial slopes and make it possible to graph the data in a compact figure, the values of the initial slopes (*s*) for the more dilute solutions have been multiplied by 40 and those for the more concentrated solutions (*s'*) by 10. Averages of *p*H values given in the table were used.

A definite relationship between the *p*H of the system and the rate of disappearance of the *o*-benzoquinone appears to exist both in the presence and the absence of unoxidized catechol. Thus if the logarithms of the initial slopes are plotted against *p*H, straight lines are obtained as shown in Fig. 2. These straight lines indicate that, within the *p*H range studied, the initial reaction slopes, or the initial rates of disappearance of the *o*-benzoquinone, are directly proportional to the hydroxide-ion concentration raised to a power, *i. e.*, $s = a[OH']^b$. Although the data at hand are not sufficient to permit any rigid conclusions in regard to this relationship, it may be pointed out that the accelerating effect of the catechol on the initial rate of disappearance of the *o*-benzoquinone appears to be at least roughly proportional to the square root of the hydroxide-ion concentration. If the logarithms of that part of the initial slope due to the accelerating effect of the catechol (slope in the presence of catechol minus slope at 100% oxidation) are plotted against the logarithm of the hydroxide-ion concentration, straight lines (three points) are obtained having slopes close to 0.5. The authors hope to investigate this more fully in the future.

Discussion

It may be stated that the present authors have not attempted to make an exhaustive study of this influence of catechol on the instability of *o*-benzoquinone. Rather, the purpose has been to gather some additional data so as to make possible a more clear interpretation of data obtained in the study of the enzymatic aerobic oxidation of catechol. In connection with the observations reported in this paper it is of interest to note that Fieser and Peters[13] in their measurements of the normal potential of the catechol–*o*-benzoquinone system at varying ratios of oxidant to reductant obtained a trend in their values which they accounted for by assuming an association between the *o*-benzoquinone and the catechol. On the other hand, Ball and Chen[10] in their measurements of the potentials of the same system reported that they observed no such trend, and that such an assumption was not necessary. They state that the value of $-\Delta e/\Delta t$, and therefore the stability of the quinone, is independent of initial concentrations of the oxidant at any given *p*H when the percentage of oxidation is 50% or less. In other words, they infer that within the ranges of concentration studied the rate of disappearance of *o*-benzoquinone is independent of the initial concentration of the *o*-benzoquinone and not influenced by the presence of catechol. The discrepancy between their findings and the results obtained in the present study, which show that catechol does exert an accelerating influence on the rate of disappearance of the *o*-benzoquinone, can possibly be accounted for by the method they employed.

According to the interpretations made by the present authors it appears that Ball and Chen varied the ratio of oxidant (*o*-benzoquinone) to reductant (catechol) at any given *p*H by varying the individual rates of flow of the oxidizing agent, ceric sulfate, and catechol into a mixing chamber. Thus as the percentage of unoxidized catechol increased (increase in per cent. reduction) the initial concentration of the *o*-benzoquinone would be progressively decreasing (total rate of flow remaining the same). Since a decrease in the initial concentration of the *o*-benzoquinone would tend to increase the stability of the latter, while an increase in concentration of unoxidized catechol would tend to decrease the stability of the *o*-benzoquinone it seems logical to suspect that these two effects might tend to compensate each other within this particular range of concentration.

Some Experimental Details.—The Valeur method[14] for determining the quinone iodimetrically was used in preference to the more recent titanium trichloride method of Knecht and Hibbert[15] or the direct thiosulfate titration method of Rzymkowski.[16] The two latter methods did not appear to be as applicable in following either the rate of the disappearance of quinone under varying conditions or the formation of the quinone in the enzymatic oxidation of catechol. Since Willstätter and Majima[17] and Rzymkowski[16] have pointed out that the accuracy of the iodimetric method is controlled by the equilibrium which is attained between the quinone, hydrogen iodide, hydroquinone and iodine, it was deemed advisable to test the method under conditions similar to those arising in this study. Aliquot samples of freshly and carefully prepared solutions of the relatively stable *p*-benzoquinone were added to molar sulfuric acid and then treated with excess potassium iodide solution. After standing in the dark for fifteen minutes they were then titrated with standard thiosulfate in the usual manner. The agreement between calculated (1.99 cc.) and average volumes of thiosulfate used (2.01 ± 0.01 cc.) was well within the estimated error of titration.

To employ this method for following the disappearance of the unstable *o*-benzoquinone according to the procedure already described, it was, of course, necessary that a negligible quantity of the quinone would be lost during the short interval of time (less than twenty seconds) between the withdrawal of the sample into the molar sulfuric acid and the addition of potassium iodide solution. It was also necessary that conditions be chosen such that the oxidation of the hydriodic acid by the *o*-benzoquinone be much faster than its own rate of disappearance in approximately 0.5 molar sulfuric acid. The good agreement between the zero time intercepts of the curves in Fig. 1 and the volumes of standard thiosulfate calculated to be equivalent to the ceric sulfate used (1.88 cc. and 5.64 cc.) indicates that this necessary condition was maintained during the course of the experimentation. The curves 5′ and 6′ in Fig. 1, representing the oxidation of hydroquinone by the same amount of ceric sulfate as employed in the more concentrated catechol experiments, also demonstrate this point, *i. e.*, the intercepts at zero time coincide with those of the catechol experiments.

It seems advisable to point out that the increased rate of disappearance of the *o*-benzoquinone in the presence of unoxidized catechol, as indicated by the increased drop in the amount of iodine liberated by the quinone, cannot be attributed to a reaction between the excess catechol and the liberated iodine. Mixtures of hydrogen peroxide and relatively large amounts of catechol liberate the same and constant amount of iodine when added to acidified potassium iodide solutions as do the hydrogen peroxide solution alone.[18] Likewise it seems improbable that the increased rate can be attributed to impurities present in the catechol. The catechol used was the chemically pure product of the Eastman Kodak Co. and had a sharp melting point. All catechol solutions were freshly prepared within a few minutes of the time they were used.

Summary

1. It has been shown that the disappearance of the unstable *o*-benzoquinone in dilute solutions can be followed by the iodimetric titration method.

2. In very dilute solutions between *p*H 4.0 and 5.5 the rate of disappearance of the *o*-benzoquinone appears to approximate a first order reaction, but as the initial concentration of the *o*-benzoquinone is increased, the rate becomes too rapid to be accounted for in this manner.

3. The rate of disappearance of the *o*-benzoquinone appears to bear a definite relationship to the hydroxide-ion concentration of the solution. This relationship appears to be of the form $s = a[OH']^b$.

4. Catechol exerts an accelerating effect on the rate of disappearance of the *o*-benzoquinone and this effect seems to be proportional to the amount of catechol present.

5. This accelerating effect of the catechol also appears to bear a similar relationship to the hydroxide-ion concentration.

6. It is most probable that in the enzymatic oxidation of catechol, the initial production of *o*-benzoquinone is followed by secondary reactions involving the *o*-benzoquinone and unoxidized catechol.

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