723. *Some Free-radical Reactions of Phenol. The Action of the Hydrogen Peroxide–Ferrous Salt Reagent and of X-Rays on Aqueous Solutions of Phenol.*

By Gabriel Stein and Joseph Weiss.

The action of OH and HO_2 radicals, produced by Fenton's reagent and irradiation with *X*-rays, respectively, on phenol in aqueous solution has been studied as a sequel to that on other benzene derivatives. Only the *o*- and the *p*-dihydroxy-derivative are formed, their relative ratios depending on the pH.

The mode of action of Fenton's reagent has been reinvestigated, with special reference to the role of the ferric ions in this system.

Under certain experimental conditions quinonoid products, and in particular *o*-benzoquinone, may be formed, apparently without the intermediate formation of quinol or catechol. This quinone obtained in *acid* solution has been shown to be similar to that obtained by the action of the enzyme tyrosinase on phenol in *neutral* solutions. Possible reaction mechanisms have been outlined.

A method for the determination of small quantities of catechol and quinol, in the presence of a large excess of phenol, and a colour reaction for *o*-benzoquinone, are described.

Further experiments on the action of mixed γ-ray–neutron radiations on benzene and on phenol under various conditions are reported.

In previous papers we have shown that free OH radicals can hydroxylate aromatic compounds. The radicals were produced by the hydrogen peroxide–ferrous salt (Fenton's) reagent and also by the action of ionising radiations on aqueous solutions (Stein and Weiss, *Nature*, 1950, **166**, 1104). The products were similar in the two cases, and it has been shown that nitrobenzene gives the isomeric nitrophenols in relative proportions which are identical within the limits of experimental error (Loebl, Stein, and Weiss, *J.*, 1949, 2074; 1950, 2704; see also *J.*, 1951, 405).

The use of the radiations to form the radicals is sometimes preferable to that of Fenton's reagent, since in the former case secondary reactions due to the metal ions are avoided and a much wider range of pH can be used. Also, Fenton's reagent can lead to a chain reaction which is often difficult to reproduce exactly, whereas when *X*-rays are used the amount of radicals formed is known and easily controlled.

We have now investigated the action of these radicals on phenol. Both the nitro- and carboxy-groups present in the compounds investigated previously (Loebl, Stein, and Weiss, *loc. cit.*) are *meta*-directing, whilst the hydroxy-group is *ortho–para*-directing. It was of interest to investigate the influence of this on substitution by a free radical (cf. Stein and Weiss, *Nature*, 1950, *loc. cit.*).

The action of Fenton's reagent on phenol has been studied previously by Martinon (*Bull. Soc. chim.*, 1885, **43**, 156) and by Goldhammer (*Biochem. Z.*, 1927, **189**, 85) amongst others.

In the classical work of Wieland and Franke (*Annalen*, 1927, **457**, 1; 1929, **475**, 1), this system served as a prototype of heavy-metal enzyme systems. Fichter and Stocker (*Ber.*, 1914, **47**, 2014; cf. Fichter and Brunner, *Bull. Soc. chim.*, 1916, **19**, 281) studied the electrolytic oxidation of phenol. Williams ("Detoxication Mechanisms," Chapman and Hall, 1947) summarised the previous work on the metabolism of phenol, and later (Porteous and Williams, *Biochem. J.*, 1949, **44**, 46, 56; Garton and Williams, *ibid.*, p. 234; 1949, **45**, 158) reinvestigated this problem. In all these cases it was found that the *meta*-isomer, resorcinol, was apparently not formed at all. However, side reactions and secondary processes made a quantitative investigation of the substitution ratios rather difficult. Baernstein (*J. Biol. Chem.*, 1945, **161**, 685), in a recent re-investigation of the biological oxidation of phenol, developed an analytical method for relatively small quantities of the hydroxylation products and has confirmed the formation of quinol and catechol. On the other hand, predominantly resorcinol was found in Barth's early work on the sodium hydroxide fusion of phenol, confirmed later by Lemberg (*Ber.*, 1929, **62**, 592), who produced evidence that this reaction presumably proceeds by way of a dehydrogenation mechanism, even in the absence of oxygen.

The Action of Fenton's Reagent.—With Fenton's reagent the conditions are somewhat complex (cf. Wieland, *loc. cit.*). The Fe^{3+} ions produced form complexes with the phenols, whilst the ferrous–ferric system enters into oxidation–reduction processes with the products. In this way some ferric iron can be again reduced to the ferrous state, thus contributing to the stationary ferrous-ion concentration in the solution. Repeating the work of previous workers, we have confirmed their results, and found that with this system reproducible results were particularly difficult to obtain. Even under conditions which led to a moderate reaction with nitrobenzene, phenol reacted violently and secondary processes, including quinone formation, obscured the final results. The secondary reactions could, however, be inhibited by a relatively small amount of ionic fluoride or pyrophosphate. In the presence of these, or of relatively large amounts of phosphate ions, only a slow reaction occurs which can be almost completely inhibited by the use of a large excess of these anions. By choice of suitable conditions, hydroxylation of phenol can then be carried out without the formation of quinones. We thus found that quinol and catechol were formed in the approximate ratio of 3 : 1 (see Table 1), with not more than 2 parts % of resorcinol, as estimated by means of colour reactions.

The action of added fluoride or phosphate consists in the elimination of the ferric ions in solution by complex formation, thus preventing the oxidation of the dihydroxybenzenes by ferric ions. On the other hand, any quinones formed by the oxidation of the dihydroxybenzenes by radicals could be reduced by the ferrous salt which, particularly in the presence of ferric complexes, would have a strong reducing action. However, the drastic inhibition of the hydroxylation reaction itself by fluoride and pyrophosphate cannot be explained on this basis alone, and it must be assumed that complex formation by the ferric ions has influenced the mechanism of the action of Fenton's reagent itself. It seems then that the role of the ferric ion in this reaction is more important than was hitherto assumed.

We have accordingly carried out experiments with ferric salts and hydrogen peroxide and have shown that aromatic hydroxylation takes place under these conditions. Similar experiments have been published in the meantime by Andersen (*Acta Chem. Scand.*, 1950, **4**, 207). These results may have one or more of several causes. It may be that ferric ions themselves can take the place of ferrous ions in the reaction leading to the hydroxylation. On the other hand it is also possible that ferric ions are necessary only as a source of a continuous supply of ferrous ions, formed, *e.g.*, by the reduction of ferric ion by HO_2^- or O_2^- (cf. Haber and Weiss, *Proc. Roy. Soc.*, 1934, *A*, **147**, 332). In the presence of a (relatively) high concentration of ferrous ion, such as may still exist initially under ordinary conditions, these would interact: $Fe^{2+} + OH \longrightarrow Fe^{3+} + OH^-$, and could very seriously compete for the available OH radicals, especially as the concentration of the aryl radicals is comparatively low; hydroxylation will then be inhibited. The retention of the OH radical in the hydroxylation process is then of fundamental importance.

It is also possible, however, that the HO_2 radical itself is capable of dehydrogenating an aromatic compound (RH): $RH + HO_2 \longrightarrow R + H_2O_2$. In this case the ferric salt–hydrogen peroxide reagent would be genuinely capable of aromatic hydroxylation. However, this reaction is about 25 kcals. less exothermic than the corresponding reaction of the OH radical because of differences in the bond energies of H–OH and H–O_2H.

These points will be discussed in subsequent publications.

It will be seen from Table I that whenever the ferric salt formed in the reaction is not removed as complex, and especially when only ferric ions are used, the formation of catechol exceeds

that of quinol. Although Baernstein's analytical procedure (which we used) involves the reduction of any quinones formed, it is still liable to give somewhat erroneous results (perhaps owing to formation of dihydroxydiphenyls, which would interfere). It therefore remains possible that the hydroxylation process is influenced by the ferric ion, possibly reacting as the $(FeOH)^{2+}$-complex (cf. Rabinowitsch and Stockmayer, *J. Amer. Chem. Soc.*, 1942, **64**, 335; Evans, George, and Uri, *Trans. Faraday Soc.*, 1949, **45**, 230; Evans and Uri, *Nature*, 1950, **166**, 869). This might occur thus: $(FeOH)^{2+} + R \longrightarrow ROH + Fe^{2+}$. Since in a reaction with a charged complex the OH radical is only implicit, different substitution ratios could result.

In addition to the possible formation of phenol in this manner, this reaction involves also the reduction of ferric to ferrous ion by the radical which is the product of the primary reaction. Therefore this reaction may also contribute to the steady state equilibrium of ferric–ferrous ions, as indicated above.

The Action of X-Rays in Aqueous Systems.—The results obtained with Fenton's reagent, although of qualitative interest, gave little quantitative information. Use of ionising radiations proved superior.

TABLE I.

Reaction of Fenton's reagent with phenol.

Initial amount of phenol: 0·5 millimole in 100 ml. of solution.

Molar ratios of phenol : H_2O_2 : Fe^{2+}	Conditions	Product formed (millimoles), quinol	catechol	Ratio, quinol / catechol	Remarks
1 : 1 : 0·25 (Fe^{3+})	Ferric ion only	0·011	0·097	0·1	Quinones formed
1 : 0·2 : 0·05	No addition	0·017	0·047	0·36	,, ,,
1 : 0·2 : 0·05	$2·5 \times 10^{-3}$ mole of NaF added	0·047	0·016	2·9	No quinone formed
1 : 0·2 : 0·05	0·12 mole of NaF added	0	0	—	Almost complete inhibition
1 : 1 : 0·5	,, ,,	0	0	—	,, ,, ,,

TABLE II.

Action of X-rays on phenol in aqueous solutions.

Dose: approx. 1×10^6 E.U.; volume 100 ml. Initial concn. of phenol: 0·5 millimole/100 ml.

pH	Conditions	Products formed (millimoles), quinol	catechol	Ratio, quinol / catechol	pH	Conditions	Products formed (millimoles), quinol	catechol	Ratio, quinol / catechol
2	Air	0·034	0·008	4·2	2	Vacuum	0·033	0·007	4·7
6	,,	0·030	0·020	1·5	6	,,	0·018	0·009	2·0
12	,,	0·024	0·009	2·6	12	,,	0·028	0·007	4·0

Baernstein's analytical method was at first used. However (cf. Table II), irradiation with the relatively large total dose of $\sim 1 \times 10^6$ E.U. (300 min. at a dose rate of 3000 E.U./min.) provided a yield which was barely sufficient for analysis and, therefore, subject to a large experimental error. Moreover, as we have shown previously (*J.*, 1949, 3245; Day and Stein, *Nature*, 1949, **164**, 671) the oxygen present in water saturated with air suffices for only a dose of approx. 6×10^4 E.U., and so irradiations with much greater doses are carried out mainly in the virtual absence of oxygen. We have used also solutions saturated with oxygen at 1 atm., but even here the oxygen suffices for only a dose of approx. $2·5 \times 10^5$ E.U.

The results shown in Table II indicated a ratio quinol : catechol of 1·5—2, in approximately neutral solution, rising to higher values in acid and alkaline media. The values obtained in the latter case were similar to those obtained by the use of Fenton's reagent, in the presence of complex-forming agents and in acid solution. Again, no resorcinol was found in the irradiated solutions.

A more sensitive analytical procedure was needed for quinol and catechol, so that smaller doses of radiation could be used. The advantage of very low doses lies also in the very low conversion of the starting material, so that the probability of any secondary attack on the primary reaction products is very small, as was proved earlier when doses of the same order were applied to aqueous solutions of nitrobenzene, benzoic acid, and chlorobenzene.—However, one should note that dihydroxydiphenyls can be formed by the interaction of the primary phenol radicals with phenol or possibly also by the dimerisation of two phenol radicals (cf. Stein and Weiss, *J.*, 1949, 3245).

A method of analysis answering our requirements consisted of the determination of (*a*) catechol by a titanium salt at a closely controlled pH and (*b*) the sum of (quinol + catechol) by Folin's reagent in acid solutions where neither phenol nor 4 : 4′-dihydroxydiphenyl interfered. The accuracy of determination of catechol was ±3%, but that for quinol (determined by difference) was only ±5—10%. Five experiments were thus used to fix each point in Fig. 1. The results show that the formation of both quinol and catechol in approximately neutral solution is a linear function of the radiation dose. In these cases the ratio quinol : catechol is about 2. The presence of hydrogen peroxide in the irradiated solution would have influenced the results to some extent: it was shown, however, that solutions of phenol, irradiated at a neutral pH, contained no more than negligible amounts of hydrogen peroxide.

FIG. 1.

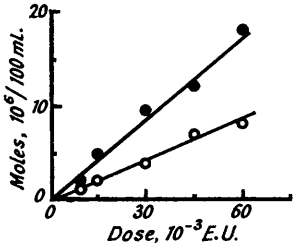


FIG. 2.

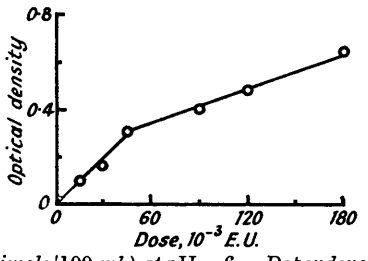


FIG. 1. *Irradiation of phenol in aqueous solution* (2 *millimole*/100 *ml.*) *at* pH ~6. *Dependence of the yield of catechol* (○) *and of quinol* (●) *on the dose.*

FIG. 2. *Irradiation of phenol in aqueous solution at* pH 2·2. *Dependence of quinone formation on the dose* (*optical density determined at* $\lambda = 290$ mμ).

FIG. 3.

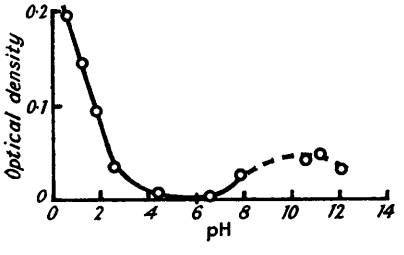


FIG. 4.

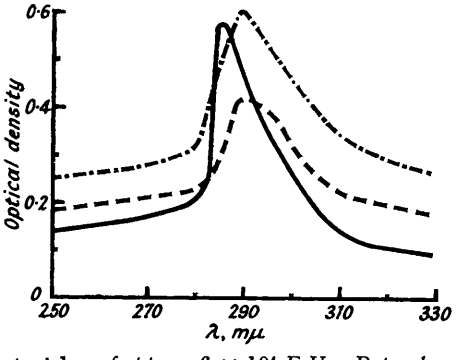


FIG. 3. *Irradiation of phenol in aqueous solution at a constant dose of approx.* 6 × 10^4 *E.U. Dependence of quinone formation on the* pH (*optical density determined at* 430 mμ).

FIG. 4. *Absorption spectra of irradiated phenol solutions. Dose approx.* 6 × 10^4 *E.U.*
———— *Irradiated at* pH 6; *spectrum determined at* pH 6.
— — — — *Irradiated at* pH 2·2; *spectrum determined at* pH 2·2.
— · — · — *Irradiated at* pH 2·2; *spectrum determined at* pH 6.

Irradiations in Acid and Alkaline Solutions.—Acid or alkaline solutions containing dissolved oxygen developed, when irradiated, a yellow or a red colour respectively. In alkaline solutions, this colour formation could not be entirely separated from the processes of autoxidation of the quinol and catechol present, which proceed readily at these pH values; nevertheless, comparison of unirradiated and irradiated alkaline samples which had been exposed to oxygen for the same length of time, showed the red colour to be due mainly to the radiation. In the acid solutions, where autoxidation does not occur, the colour was a well defined function of the radiation dosage (Fig. 2); incidentally acid solutions of phenol containing quinol and catechol remained colourless unless irradiated. The colour formed in the irradiated acid solutions could be discharged by adding sulphur dioxide to the acid or after neutralised solution; thereafter quinol and catechol were shown to be present, the ratio being about 4. Acid solutions of phenol irradiated in the absence of air remained colourless, but again the ratio was about 4.

The coloured substances are possibly quinonoid. Colour formation is strongest at the low pH values (see Fig. 3), disappears completely at pH ~4—7, and reappears again at more alkaline pH. The reappearance is genuine and occurs under conditions where autoxidation is not yet very noticeable. The experiments could not be followed, however, to higher pH values because from pH ~11 the red (quinonoid) product changes spontaneously, in a fast reaction, into another product with a different absorption spectrum (its maximum is at approx. 310 mμ and the optical density is lower at 430 mμ). The spectra of the irradiated phenol solutions are shown in Fig. 4, being obtained from the difference between the unirradiated and irradiated solutions. " Neutral " solutions of phenol (buffered or unbuffered) show, after irradiation, a spectrum in the near ultra-violet which is consistent with a mixture of quinol and catechol. Acid solutions of phenol show, after irradiation, a spectrum which indicates the presence of quinones. If an irradiated acid solution is gradually neutralised, the wave-length of maximum absorption is unchanged, but the general light absorption in-

FIG. 5.

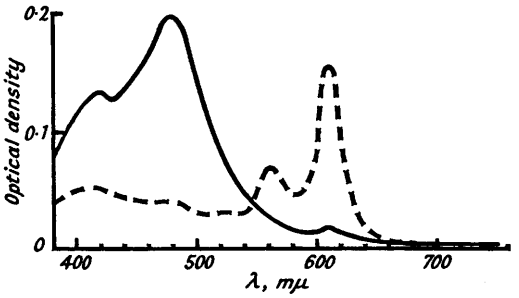


FIG. 6.

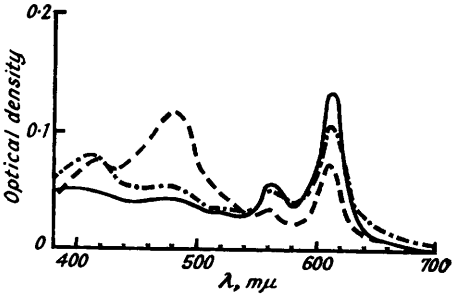


FIG. 5. *Absorption spectra of the products of reactions of* p- (————) *and of* o-*benzoquinone* (— — — —) *with* o-*phenylenediamine.*

FIG. 6. *Absorption spectra of the reaction products of* o-*phenylenediamine with* (————) *phenol solution irradiated at* pH 0·6, (— · — · —) *phenol solution treated with tyrosinase at* pH 7, *and* (— — — — —) *a solution of* p-*benzoquinone in acid, to which catechol has been added.*

creases; this increase is proportional to the pH, as shown in Table III; this behaviour is similar to that of, *e.g.*, quinhydrone or phenoquinone, but does not correspond entirely to any of the derivatives of *p*-quinone which we have tested.

TABLE III.

Dependence of optical density on pH *after irradiation.*

(Solutions of phenol irradiated at pH 1·2 with a dose of approx. 6×10^4 E.U. Results obtained with two different solutions for ascending and with one solution for descending pH. Optical density determined at ~430 mμ, Ilford filter No. 601.)

pH	Optical density (x)	10^2x/pH	pH	Optical density (x)	10^2x/pH
1·75	0·077	4·40	8·00	0·353	4·42
2·00	0·086	4·30	6·15	0·256	4·17
2·30	0·098	4·26	5·70	0·245	4·30
			5·45	0·236	4·33
2·60	0·126	4·84			
3·10	0·145	4·67			
3·85	0·174	4·52			
4·95	0·203	4·10			

Reaction with aniline (Pugh and Raper, *Biochem. J.*, 1927, **21**, 1375) gave positive results for the presence of an *o*-quinone but the yields were insufficient for isolations of solid derivatives. Following a suggestion by Dr. D. G. I. Felton we found that *o*-phenylenediamine and authentic *o*-benzoquinone in acid solution gave a brilliant green solution with an exceptionally strong red fluorescence noticeable even at approx. 0·1 micromole/ml. Solutions containing *p*-benzoquinone or its derivatives gave non-fluorescent red solutions. Fig. 5 shows the spectra of the products of reaction of *o*- and *p*-benzoquinone with *o*-phenylenediamine in acid solution.

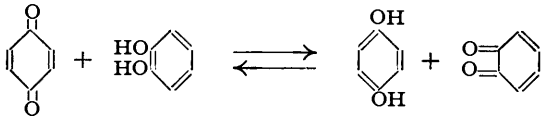
Solutions of phenol irradiated at or below pH ~1 give, after treatment with *o*-phenylenediamine, spectra identical with those of *o*-benzoquinone (Fig. 6).

These results indicate that irradiation of acid phenol solutions gives an *o*-quinone. It is of

interest that Pugh and Raper (*loc. cit.*) found that the action of the enzyme tyrosinase on a neutral solution of phenol yields an *o*-quinone. We have repeated this experiment using tyrosinase prepared from mushrooms, and a 0·2% solution of phenol at pH ~7. After 30 minutes at room temperature the reaction mixture was acidified and treated with *o*-phenylenediamine. The spectrum of the resulting solution was identical with that of *o*-benzoquinone, or that of the irradiated phenol solution (Fig. 6).

The substance present in these irradiated solutions, which thus appears to be an *o*-quinone, must co-exist with an excess of quinol (cf. Fig. 3, and results after reduction, Experimental section). The question arises whether these can be in a stable equilibrium in solution.

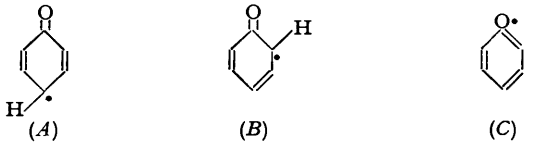
We find that *o*-benzoquinone does not react with quinol in acid solution: the spectrum of the product obtained by adding *o*-phenylenediamine to this mixture is identical with that obtained from *o*-benzoquinone. On the other hand, *p*-benzoquinone reacts with catechol in acid solution: the *o*-phenylenediamine test shows the presence of *o*- as well as of *p*-benzoquinone (Fig. 6). This may appear somewhat surprising, as the normal potential of *o*- is slightly higher than that of *p*-benzoquinone (by approx. 0·09 v according to Conant and Fieser, *J. Amer. Chem. Soc.*, 1924, **46**, 1858). In this system, however, one deals with the equilibrium:



The state of this equilibrium will depend not only on the standard free energies of the separate components, but also on the free-energy changes due to the corresponding quinhydrones, which may be any of three or possibly four different combinations. Thus, the free-energy change due to these may possibly exceed the small difference in the oxidation potentials of the quinones that may still exist under the conditions employed. It appears then, that in acid solutions the equilibrium is shifted towards a system in which the *o*-quinone is capable of existence.

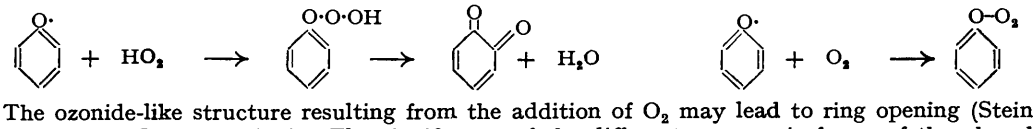
As to the mode of formation of this quinone, we have already excluded the possibility that it is the product of the autoxidation of quinol or catechol, which are primarily formed. There is also the possibility that quinol and catechol, once formed, could be further oxidised by the radiation, even in the presence of a large excess of phenol. This is unlikely, however, for the following reasons: the addition of a small quantity of quinol and catechol (such as would be formed by the irradiation) to a solution of phenol before irradiation does not increase the yield of the quinone. Also Fig. 2 shows that the formation of the quinone is linear with the dose, even at very low doses, and does not show an induction period for the formation of the diols. Fig. 3 moreover shows that in neutral solutions, where more quinol and catechol are formed and where both quinol and catechol are inherently easier to oxidise, quinone formation does not occur. Fig. 3 rather suggests that the formation of the quinone depends on some intermediate entity which is subject to a pH-dependent equilibrium.

All these results indicate that the quinone is formed not from, but in place of, quinol and catechol. The reaction may occur in the following stages: phenol is dehydrogenated by a free radical to yield the free phenol radical, which can, however, exist in several forms, *e.g.*, (*A*—*C*). Some of these forms are explicitly quinonoid (cf. Wheland, *J. Amer. Chem. Soc.*,



(*A*) (*B*) (*C*)

1942, **64**, 900) and could yield the observed quinol: catechol ratio and exclusively *ortho–para*-substitution. Structure (*C*), moreover, may result directly by dehydrogenation of phenol, and it is possible that in strongly acid solutions this point of dehydrogenation will compete with dehydrogenation in the ring. This structure may yield *o*-quinone, *e.g.*, according to the schemes:



The ozonide-like structure resulting from the addition of O_2 may lead to ring opening (Stein and Weiss, *J.*, 1949, 3254). The significance of the different mesomeric forms of the phenol

free radical has been pointed out recently by Cosgrove and Waters (*J.*, 1951, 388). Also, at different pH values different mechanisms may operate for the decomposition of the peroxide under consideration. The role of similar structures has been already considered for cresols by Pummerer, Puttfarcken, and Schopflocher (*Ber.*, 1925, **58**, 1808) and by Westerfeld (*J. Biol. Chem.*, 1942, **145**, 463). Somewhat similar considerations have been employed also by Saunders and Watson (*Biochem. J.*, 1950, **46**, 629) in describing the action of peroxidase, whilst a shift to a quinonoid structure in both acid and alkaline media is described by Witkop and Patrick (*Experientia*, 1950, **6**, 183). In our view, under the experimental conditions employed in the present work, oxidation of phenol to a quinonoid product occurs without the intermediate formation of a dihydroxybenzene.

The quinonoid product which predominated in our experiments was an *o*-quinone. The very interesting fact emerges that the free-radical oxidation of phenol, *in vitro*, at an acid pH leads to the same product as the action of tyrosinase at a *neutral* pH. The function of the acid environment in our case appears to be to ensure the stability of one particular intermediate. Similarly one role of the enzyme surface could be to stabilise one particular form of the radical by providing a suitable environment, corresponding to a lower pH.

This view is supported by the work of Kuhn and Wagner-Jauregg (*Ber.*, 1934, **67**, 361) and of Haas (*Biochem. Z.*, 1937, **290**, 291) who showed that the semiquinone radical of lactoflavine which was stable *in vitro* only in acid solutions became stable in neutral solutions on the surface of the protein, in combination with which it forms the enzyme (" yellow ferment ").

Further Experiments on the Action of Neutrons on Benzene and Phenol in Aqueous Solutions.—In previous papers (Stein and Weiss, *Nature*, 1948, **161**, 650; *J.*, 1949, 3254) we have reported some preliminary experiments with a mixed γ-ray–neutron source, with aqueous solutions of benzene in a vacuum. We found quinol and catechol, in addition to phenol. Using dinitrophenylhydrazine as a reagent, we isolated a product which appeared to be a dialdehyde formed by opening of the benzene ring. In view of the apparent connections with the present work, we have carried out some further experiments.

The only source of neutrons at our disposal was a 1-g. Ra–Be source, giving approx. 10^{10} γ-ray quanta and approx. 10^6 neutrons per second, so that only a small fraction of the energy absorbed in the irradiated solution is due to the neutrons.

Using the colour reaction described above we confirmed our earlier finding that, with a constant γ-ray intensity, the yield was approximately twice as great when the mixed radiation was used. In view of the low neutron intensity, this was remarkable; it resembles the results of Hopwood and Phillips (*Nature*, 1935, **136**, 1026; 1939, **143**, 640). If the irradiations are carried out in the presence of oxygen, this difference between the yields from γ and γ–n sources disappears (Dr. M. Ebert, personal communication; we have confirmed this result). Thus, it is possible that the cause of the difference in the experiments in a vacuum is the formation of oxygen by neutrons. If so, the effect would at least qualitatively be similar to the differential effects observed between densely and sparsely ionising radiations (Bonét-Maury and Lefort, *Nature*, 1948, **162**, 381; Haissinsky and Lefort, *Compt. rend.*, 1949, **228**, 314). Irradiating a neutral solution of phenol with *X*-rays or with the mixed γ-ray–neutron source in the presence of air and treating the solution with dinitrophenylhydrazine gives a precipitate which appears to be the same as that obtained from benzene (*loc. cit.*). By an improved method of elution chromatography, we have now separated this substance into three components. The largest part consists of a dinitrophenylhydrazone, m. p. 300—305°, which appears to be a derivative of an aliphatic dialdehyde and is obtained by irradiation of benzene or of phenol. Paper chromatography with ethanol–butanol–ammonia solution confirmed the identity of the two substances, whilst a solution of phenol, irradiated at an acid pH, yielded a precipitate with dinitrophenylhydrazine, which behaved differently from those obtained from neutral solutions and appears to be a derivative of a quinone.

Experimental.

Materials.—Phenol. This was AnalaR material (Messrs. Hopkin and Williams). Unless otherwise stated, a solution of 2 millimoles of this material in water (100 ml.) was used at a pH ~6 in the irradiations, and one of 0·5 millimole/100 ml. in the experiments with Fenton's reagent.

Water. For the experiments with Fenton's reagent, and for the irradiation with large doses, ordinary distilled water was used. For irradiations with low doses triple-distilled water was used, ordinary distilled water being redistilled in an all-glass still from alkaline permanganate and then from phosphoric acid.

4 : 4′-*Dihydroxydiphenyl.*—This was prepared by Hirsch's method (*Ber.*, 1899, **22**, 335). Recrystallised from dilute sulphuric acid it had m. p. 273—274° (Schmidt and Schultz, *Annalen*, 1881, **207**, 334, give m. p. 272°).

Catechol. This was purified by conversion into the lead salt, decomposition thereof by hydrogen sulphide, extraction with ether, and one recrystallisation from triple-distilled water.

Quinol. Obtained from Messrs. Hopkin and Williams, this was not further purified.

o-*Benzoquinone.* This was prepared by Willstätter and Müller's method (*loc. cit.*).

Experiments with Fenton's Reagent.—The ferrous and ferric sulphates used were of AnalaR quality (Messrs. Hopkin and Williams). The hydrogen peroxide was 90% material, free from stabilisers, suitably diluted. Experiments were carried out as described previously (Loebl, Stein, and Weiss, *J.*, 1949, 2074). Separate solutions of hydrogen peroxide and ferrous or ferric sulphate were run simultaneously into a stirred solution of acidified phenol. As complex-forming agents phosphoric acid (AnalaR) and sodium fluoride (puriss.; Messrs. Hopkin and Williams) were used.

Irradiations.—These were carried out as described previously (Farmer, Stein, and Weiss, *J.*, 1949, 3241). A Victor Maximar set was used at 200 kv and 15 mA, without filtration. The dose, determined according to Day and Stein's method (*Nature*, 1949, **164**, 671; *Nucleonics*, 1951, **8**, No. 2, p. 34) was approx. 2300 E.U./min. when 200 ml. of the solution were irradiated, and approx. 3200 E.U./min. when 100 ml. solution were irradiated. At the wave-length of the *X*-rays used, 1 E.U. equals approximately 1 roentgen. Experiments were carried out (*a*) in air, (*b*) in a vacuum (the solution being freed from air by passage through it of purified nitrogen, followed by evacuation), or (*c*) in solutions saturated with oxygen (oxygen being bubbled through the solution for at least 30 minutes before irradiation). The pH values stated in the experiments were maintained by the addition of sulphuric acid (AnalaR) for pH values lower than 3, by phosphate buffers (Na_2HPO_4 and KH_2PO_4, AnalaR) between pH 3 and 8, and by sodium hydroxide (AnalaR) above pH 8. Phosphate ions do not interact with the radicals.

Detection of Resorcinol (cf. Krauskopf and Ritter, *J. Amer. Chem. Soc.*, 1916, **38**, 2182).—To synthetic mixtures containing phenol, catechol, and quinol in amounts similar to those obtained in experiments with Fenton's reagent or with large doses of irradiations, were added 4 mg. of cobalt chloride and various amounts of resorcinol, the resulting mixture being diluted to 100 ml., to which 2 ml. of ammonia solution (*d* 0·88) were added. Transient intense green coloration, changing to brown, was obtained, the lowest resorcinol concentration yielding a definite result being 0·001 millimole/100 ml., in the presence of 0·5 millimole of phenol. In experiments with Fenton's reagent or *X*-rays, even when the amount of catechol + quinol amounted to 0·05 millimole or more, no positive reaction for resorcinol was obtained. If to such solutions 0·001 millimole or more of resorcinol was added the colour reaction was positive.

Determination of Catechol and Quinol.—Method 1. This method was essentially that of Baernstein (*loc. cit.*). In experiments with Fenton's reagent, ether-extraction was continued for 16 hours. In radiation experiments extraction was unnecessary and the determinations were carried out directly on the reaction mixture. In both cases sodium sulphite was added before analysis. Catechol was determined by lead acetate at pH 6·5 in the presence of a pyridine buffer, the precipitate being filtered off in a sintered-glass crucible and then dissolved in acetic acid; the lead was precipitated as iodate, and IO_3^- determined in this precipitate by the liberation of iodine from potassium iodide, and titration against 0·2N-sodium thiosulphate. In the filtrate of the first (catechol) precipitate, the quinol, and subsequently the phenol, was determined by iodination at fixed pH values, potassium iodide and a bromide–bromate solution being used. The liberate diodine was titrated as above, by a potentiometric method, with a platinised platinum electrode and a dip-type calomel electrode, in conjunction with a Cambridge pH meter set for E.M.F. measurements. Readings of the E.M.F. (*E*) were taken for increments (*V*) of 0·1 ml. of titrant in the vicinity of the end point, and the end point was determined graphically from a plot of dE/dV against *V*. Other particulars of the method, preparations of the standard solutions, and general procedure were as in Baernstein's paper (*loc. cit.*). Control analyses on synthetic solutions containing phenol, catechol, and quinol showed that the method was accurate within ±4%, when the amounts present were of the order of 0·1 millimole/100 ml., but the procedure is very tedious. 4 : 4′-Dihydroxydiphenyl, when present, interfered, leading to "recoveries" of phenol greater than the amount originally present, but apparently it did not interfere with the determination of quinol or catechol.

Method 2. (i) The reagent solution was prepared immediately before use by adding 2 ml. of 15% titanium(III) chloride or sulphate solution to 5 ml. of 2N-sulphuric acid, and diluting the whole to 200 ml. with water. An aliquot of the neutral, irradiated solution was made up to exactly 50 ml. and transferred to a 100-ml. measuring flask. To this was added 5 ml. of a disodium hydrogen phosphate solution (10 g. of anhydrous salt in 100 ml. of water) and then, with constant stirring, drop by drop, 2 ml. of the freshly made reagent; the pH of the mixture was then 7·9—8·0. After 30 minutes the solution was made up to 100 ml. and the intensity of the yellow colour measured. Phenol, quinol, and 4 : 4′-dihydroxydiphenyl do not interfere. Hydrogen peroxide interferes to some extent and must be absent for accurate determinations. A calibration curve was obtained by using a Spekker colorimeter, Ilford filter 601, and a 3-cm. cell. Full optical density was reached at room temperature only after about 30 minutes, but the process could be accelerated by warming. We did not do so, since the simultaneous determination with Folin's reagent also took about 30 minutes. Optical density was a linear function of the concentration of catechol in the range 1—7 micromoles/100 ml. For the colour formation of catechol with Ti^{3+}, cf. Piccard (*Ber.*, 1909, **42**, 4343).

(ii) The amount of (quinol + catechol) in the solution was determined by Folin's reagent at an acid pH, and not in alkaline solution as usual. An aliquot of the irradiated solution was made up to 25 ml. with water and transferred to a 100-ml. measuring flask. To this was added 5 ml. of a solution of potassium dihydrogen phosphate (10 g. in 100 ml. of water), and then exactly 1 ml. of Folin–Ciocalteau's reagent. This reagent was prepared by treating sodium tungstate (100 g.) and molybdate (25 g.) with water (700 ml.), 85% phosphoric acid (50 ml.), and concentrated hydrochloric acid (100 ml.), boiling the whole gently with reflux for 10 hours, adding lithium sulphate (150 g.), water (50 ml.), and bromine (a few drops), then boiling the mixture without a condenser for 15 minutes in a fume cupboard, cooling it,

diluting it to 1 l., and filtering it through a fritted-glass filter after 3 days (cf. Snell, " Colorimetric Methods of Analysis," 1937, Vol. II, p. 197).

After addition of the reagent, the pH of the test solution was 2·6—2·7. The mixture was then immersed into a boiling-water bath for exactly 30 minutes and diluted immediately afterwards with cold water to 100 ml.; the intensity of the colour was then measured; as comparison solution an unirradiated phenol solution treated in the same manner was used. Under these conditions phenol and 4 : 4'-dihydroxydiphenyl do not interfere. Hydrogen peroxide interferes only to a small extent, when present in comparable quantities.

(iii) The calibration curves for the determination of catechol and of quinol by Folin's reagent (Spekker colorimeter, Ilford filter 608, 3-cm. cell) show that the optical density is a linear function of the concentration of catechol up to about 1 micromole/100 ml., and also a linear function of the concentration of quinol in the range 1—5 micromoles/100 ml. The volume of the irradiated solution used in one test was 2—3 times greater for the determination of catechol by Ti^{3+} than the volume used in the Folin test. Fig. 7 shows the influence of temperature and time of heating on the optical density obtained. The annexed Table shows some results obtained on synthetic mixtures.

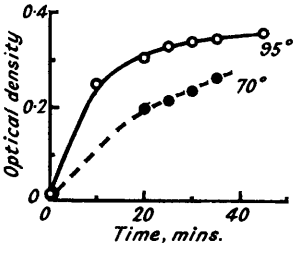


FIG. 7.

Influence of time and temperature of heating on the colour development in the determination of catechol by Folin's reagent.

Analysis of synthetic mixtures of quinol and catechol by method 2 (quantities in micromoles/100 ml.).

Calculated		Found	
Quinol	Catechol	Quinol	Catechol
1·70	0·355	1·45, 1·6, 1·7, 1·5	0·35, 0·34, 0·36, 0·35
0·85	0·355	0·7, 0·8, 0·75	0·36, 0·355, 0·345

Detection of Hydrogen Peroxide.—To ascertain the presence or absence of hydrogen peroxide in the irradiated solutions, tests described by Feigl (" Qualitative Analysis by Spot Tests," 1939) were used. Even in the presence of catechol, that employing an acid solution of a Ti^{3+} salt can be used. We have mainly relied on the sodium carbonate–cerium(III) sulphate test. The reagent is nearly colourless and gives a strong red-to-brown colour with traces of hydrogen peroxide. With the unirradiated phenol solution and with the same solution after irradiation at a neutral pH, this gave a greyish precipitate, but no red colour, whilst if a trace of hydrogen peroxide were added to the solutions, the red colour reaction could be observed, not masked by the reaction with phenol. Whilst no peroxide was thus found to be present in neutral irradiated solutions, a weak positive reaction was obtained with irradiated acid solutions.

Ratio of Quinol : Catechol in Solutions irradiated at an Acid pH.—A 0·2% solution (100 ml.) of phenol was irradiated at pH 2·2, and then treated with dilute aqueous sulphur dioxide drop by drop, till all the colour disappeared, and then 1 drop was added in excess. Any excess of sulphur dioxide interferes in the determination by Folin's reagent, and it was therefore driven from the solution by evacuation and boiling out at room temperature, or alternatively by aeration at 50°. Owing to this procedure, the results were not very accurate for quinol, but quite reliable for catechol. For a dose of 6×10^{4} E.U. the amount of catechol found was $(3{\cdot}4 \pm 0{\cdot}2) \times 10^{-6}$ mole, whilst the yield of quinol was $2—3 \times 10^{-5}$ mole, the ratio varying from 5 : 1 to 8 : 1. Comparison of these results with those shown in Fig. 1, obtained in neutral solutions, shows that the yield of catechol has decreased and that of quinol increased. This is in qualitative agreement also with the results obtained by Baernstein's method on solutions irradiated with higher doses, as shown in Table II. Reduction with sulphur dioxide gives the corresponding dihydroxybenzene from quinones. Whilst this is likely to be so in the present case, we have no independent proof, that the quinonoid product, after reduction, appears in the form of catechol or quinol, respectively. However, the results of experiments carried out in a vacuum (below) seem to support this.

Irradiations in a Vacuum.—A few experiments were carried out to determine the yield of hydrogen in irradiations in a vacuum. The apparatus used for evacuation, irradiation, and analysis were as described previously (Farmer, Stein, and Weiss, *loc. cit.*). When 100 ml. of a neutral 0·2% solution of phenol were irradiated with a dose of $3{\cdot}6 \times 10^{5}$ E.U. the yield of hydrogen (calc. in terms of H atoms, cf. Stein and Weiss, *J.*, 1949, 3245) was $6{\cdot}2 \times 10^{-5}$ equivalent, whilst a similar irradiation carried out at pH 2·2 yielded only $2{\cdot}4 \times 10^{-5}$ equivalent. Both the neutral and the acid solution remained colourless after irradiation, showing that, in the absence of oxygen, quinone formation could not take place. In a vacuum, the aryl radicals may react with a molecule of phenol or another radical (Stein and Weiss, *loc. cit.*).

Experiments in a vacuum (where quinone formation does not occur) yielded values for catechol and quinol in acid solution (Table II) which are in qualitative agreement with those obtained in the presence of oxygen (see previous section). The reduced yields of hydrogen in acid solutions indicate perhaps the presence of aryl radicals, which react with hydrogen atoms more rapidly than do the aryl radicals present in neutral solution, thus reducing the number of hydrogen atoms which can yield hydrogen molecules.

Absorption Spectra and Colorimetric Measurements.—The ultra-violet and visible absorption spectra shown in Figs. 4, 5, and 6, as well as the results shown in Fig. 2, were obtained by using a Unicam S.P. 500 instrument, and appropriate silica or glass cells. Fig. 3 was obtained with a Spekker colorimeter with Ilford filter No. 601, having a maximum transmission at approx. 430 mμ.

Fig. 2 was obtained by taking, at the pH at which the irradiation was carried out, the complete spectra of solutions irradiated at pH 2·2 with the doses stated, and plotting the optical densities obtained at $\lambda_{max.}$, *i.e.*, 290 mμ. Fig. 3 was obtained by irradiating the solutions at the pH values stated with a constant dose of 6×10^4 E.U. and then bringing every solution to a pH value of $5{\cdot}5 \pm 0{\cdot}1$ (controlled with a Cambridge pH meter and a glass electrode) before determination of the optical density. The curves shown in Fig. 4 were obtained by measuring the unirradiated against the irradiated solution in the Unicam instrument.

Colour Reactions with o*-Phenylenediamine.*—To the acid solution containing *o*-benzoquinone (extracted from the original ethereal solution) there was added 1 ml. of a saturated solution of *o*-phenylenediamine in 2N-sulphuric acid. The concentration of the quinone affected the time for formation of the green colour and appearance of the red fluorescence. This process may be accelerated by gentle warming. The formation of the red colour with *p*-benzoquinone is faster. Neutralising the *o*-quinone–diamine solution causes the fluorescence to disappear, the colour to change to yellow, and a precipitate to appear. From concentrated reaction mixtures a precipitate appears even in acid solutions. These products are being investigated.

Enzymic Experiments.—Tyrosinase was prepared from mushrooms by Mr. F. K. Duxbury by Jensen and Tenenbaum's procedure (*J. Biol. Chem.*, 1942, **145**, 293; 1943, **147**, 737). The tyrosinase solution (1 ml.) was added to 0·2% phenol solution (100 ml.) in the presence of a phosphate buffer at pH 7. After 30 minutes at room temperature, the solution was made N. with sulphuric acid, and then the colour test with *o*-phenylenediamine was carried out, the spectrum of the resultant solution being shown in Fig. 5.

Experiments with Mixed Neutron–γ *Rays.*—These were carried out as described previously (Stein and Weiss, *J.*, 1949, 3254). The mixture was separated by elution chromatography on alumina. The presumed dialdehyde derivative was eluted in the 1 : 1 light petroleum–benzene fraction. Recrystallised from light petroleum, it had m. p. 300—305° (preheating to 290°) (cf. Baker, Ollis, and Zeally, *J.*, 1951, 206). Acid solutions of phenol, when irradiated, did not yield this product in large quantities, but gave instead a product which was extremely strongly adsorbed in alumina. This dinitrophenylhydrazone changes colour on treatment with ammonia, as do derivatives of acids. Dinitrophenylhydrazones, *e.g.*, that of *p*-benzoquinone, behave similarly. On paper chromatography the product obtained from the irradiated acid solution (Whatman filter paper No. 1, butanol–ethanol–ammonia solution) behaved quite differently from the derivatives obtained by irradiation of benzene or phenol in neutral solution, and exhibited R_F values similar to those of quinone derivatives. We hope to investigate these points further.

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