LXXII. THE BACTERICIDAL ACTION OF THE QUINONES AND ALLIED COMPOUNDS.

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Wurster [1889] and Raciborski [1907] first showed that various quinones gave red colorations with proteins, proteoses, and amino-acids. Meunier and Seyewetz [1908] furthermore showed that hide could be tanned by quinol in the presence of oxygen and alkali or by benzoquinone itself, and suggested that the tanning process was due to the reaction between this quinone and the amino-groups of the tissue. Fischer and Schrader [1910] found that qiunones reacted with ethylglycine with the formation of quinol and a substance analogous to di-anilinoquinone.

One of us [Cooper, 1913] confirmed the observations of Wurster and Raciborski and investigated in more detail the relations of *p*-benzoquinone to proteins. It was found that not only were proteins coloured permanently red by treatment with solutions of this quinone, but other physical properties, *e.g.* solubility and precipitability, were also changed, and evidence was adduced that the quinone reacted as in the case of formaldehyde with the amino-groups of the proteins. Shortly afterwards Scharvin [1913] also observed that various proteins were dyed by benzoquinone and its homologues, and came to the same conclusion as the former workers in regard to the nature of the dyeing process. Anthraquinone and phenanthraquinone were without action.

Suida [1913] also found that wool was dyed by solutions of benzoquinone, toluquinone and 3:6-*o*-xyloquinone in acetic acid solution, but not by 2:5-*p*- and 2:5-*m*-xyloquinones, thymoquinone, anthraquinone or phenanthraquinone. He pointed out that the quinones active in dyeing all contained the —CO—CH=CH—CO— group and concluded that they reacted with the amino-constituents of the wool.

These facts themselves suggest that quinones would be powerful germicides and Thalhimer and Palmer [1911] found (apart from these considerations) that benzoquinone greatly exceeded phenol, cresol, quinol, and other substances in germicidal power, this being confirmed by Cooper [1912, 1].

A further study of the efficacy of the quinones and their derivatives as disinfectants has since been made with the object of discovering substances

suitable for use as germicides in the living organism, and the results so far obtained are set out in the present paper. First of all, however, reference should be made to certain observations of theoretical interest, bearing on the mechanism of the reaction which proceeds between quinones and proteins.

I. The Relations of Quinone to Proteins.

(*a*) *General Observations.*

As stated in a previous paper [Cooper, 1913] when gelatin was immersed in aqueous *p*-benzoquinone solutions, it became permanently red and insoluble in water. Egg-albumin suspensions also gave a red coloration, but the protein was not precipitated. Subsequently, however, it was found that if the albumin solution were mixed with strong solutions of this quinone (0·5 to 1 %) and the mixture left for several days, a red flocculent nitrogenous precipitate separated. This product was insoluble even in large volumes of water and alcohol, and was not decolorised by boiling in either medium. Unlike albumin coagulated by heat, or phenol, the precipitate was insoluble in hot anhydrous phenol or *m*-cresol and in acid, and was only slowly dispersed by alkali. The dispersion was destroyed by the addition of acid and by dilute ammonium sulphate, saturated solution of magnesium sulphate and sodium chloride, as is the case with albumin denaturated by heat or phenol.

The effect of some of the homologues of benzoquinone has also been studied. Toluquinone also gave intense red colorations with gelatin, egg-albumin, and caseinogen; xyloquinone and thymoquinone gave however no colorations.

Equal amounts of washed dry gelatin were immersed at room temperatures in equimolecular solutions of *p*-benzoquinone, toluquinone, xyloquinone, and thymoquinone (0·023, 0·037, 0·04, 0·05 % respectively). Immersion for less than 24 hours in the benzoquinone solution was sufficient to render the gelatin intensely red and insoluble in water. The gelatin suspended in the toluquinone solution for the same length of time was coloured less intensely and even after six weeks was not rendered insoluble. The gelatin immersed in the solutions of the higher homologues was unaffected. From these observations it seems that as the homologous series is ascended the reactivity of the quinones towards proteins diminishes; in the case of the phenols and alcohols, on the other hand, it was found [Cooper, 1913] that their protein-precipitating power increased as successive homologues were considered.

(*b*) *The removal of quinone from solution by proteins.*

The experiments described in this section were carried out with the object of investigating the influence of concentration and other factors upon the amount of quinone removed by proteins from aqueous solution.

Materials used. The proteins used were gelatin, egg-albumin and caseinogen.

Gelatin. The Swiss Gold Label brand was used. The gelatin was washed in running water for 24 hours, drained, air-dried and then cut into small slips and dried at 110°.

Egg-albumin. Crystalline egg-albumin prepared by the method of Hopkins and Pinkus [1898] was used. The crystalline protein was dialysed in running water and employed as a colloidal suspension.

Caseinogen, prepared according to Hammarsten, was employed. It was dried at 110° before use.

Estimation of quinones. The method used, which was that devised by Valeur for *p*-benzoquinone, and its homologues and derivatives, depends on the fact that a quinone sets free iodine from a mixture of hydrochloric acid and potassium iodide.

Experimental Methods.

Definite amounts of a protein were immersed in equal volumes of quinone solutions of known strength. At stated times the amounts of quinone left in the solutions were again estimated.

(*a*) *Experiments with precipitated caseinogen.*

The dialysing method of Moore and Bigland [1910] was employed, the protein being restricted to one region of the water-phase by means of parchment paper, so that the content of quinone in the aqueous solution could be accurately determined.

It was found that the quinone dialysed rapidly and was not taken up by the parchment. With phenol under these circumstances equilibrium was attained at 20° within 40 hours [Cooper, 1913], but with quinone, the concentration in the water-phase continued to fall after a much longer period and equilibrium was not reached in 10 days. For this reason *dispersed* proteins were subsequently used.

(*b*) *Egg-albumin (colloidal solution).*

Ten cc. of a dialysed suspension (18 %) were placed within the dialysing bag at 20° and 25 cc. of benzoquinone solution outside.

Table I.

Initial strengths of quinone solution			1. 0·284 %
,,	,,	,,	2 0·284
,,	,,	,,	3. 0·192
,,	,,	,,	4. 0·192

The concentration of quinone continued to fall during four weeks, until by the 30th day no quinone could be detected. In the case of phenol, however, the maximum amount was absorbed by the protein within 24 hours [Cooper, 1912, 1] in accordance with the partition-law. From the above results it is found that 1·8 g. of albumin removed 0·099 and 0·050 g. of quinone respectively from the solutions of 0·284 and 0·192 %. The amounts of phenol absorbed by

the same weight of protein from the same initial concentrations were 0·012 and 0·006 g. [Cooper, 1913], (calculated from partition-coefficients). Quinone was thus removed by egg-albumin in much greater amount than phenol from the same concentration.

(*c*) *Gelatin.*

As at 20° gelatin is not appreciably soluble in water, in the following experiments the dialyser was dispensed with.

Table II.

Weight of protein 2 g. Volume of quinone solution 100 cc. 20°.

Initial concentrations	1.	1·04 %
„ „	2.	0·52
„ „	3.	0·26

A fall in quinone concentration was again observed to continue for several weeks, and at the end of 50 days no quinone was left in solution. The uptake of phenol by gelatin however was complete within two minutes [Cooper, 1913].

The results show that 2 g. of gelatin removed 1·04, 0·52, 0·26 g. of quinone respectively from the three solutions. The amounts of phenol removed by the same amount of protein from the same concentrations of phenol, on the other hand, were 0·06, 0·03, 0·015 g. respectively [Cooper, 1913], (calculated from partition-coefficients). As in the case of albumin, more quinone than phenol was thus removed by gelatin from the same concentration of these substances. Furthermore, while the absorption of phenol by gelatin and albumin was reversible, it was not possible to recover any quinone from the proteins even after boiling in water.

Experiments were next carried out with smaller amounts of gelatin with the object of determining an equilibrium-point.

Table III.

Initial quinone concentration	Quinone concentration in water-phase after 8 weeks	Total amount of quinone removed by the protein after 8 weeks
(*a*) Weight of gelatin 0·5 g. Vol. of quinone solution 150 cc. Temp. 20°:		
1. (*a*) 0·620 %	0·198 %	0·633 g.
(*b*) 0·620	0·198	0·633
2. (*a*) 0·310	0·106	0·306
(*b*) 0·310	0·106	0·306
3. (*a*) 0·124	0·033	0·136
(*b*) 0·124	0·033	0·136
4. 0·062	0·009	0·080
(*b*) Weight of gelatin 0·25 g.		
1. 0·620 %	0·320 %	0·450 g.
2. (*a*) 0·310	0·176	0·201
(*b*) 0·310	0·176	0·201
3. 0·124	0·062	0·093
4. 0·062	0·029	0·050

From all the foregoing concentrations the gelatin continued to remove quinone for several weeks, and equilibrium was not even attained after

eight weeks. It is seen from the table that gelatin removed or reacted with very large amounts of quinone, 0·25 g. of the protein for example removing from an initial concentration of 0·62 % as much as 0·45 g. of quinone. The results in eight weeks also show that the amount of quinone disappearing depends upon the initial concentration, being in fact approximately proportional to the concentration.

The observations so far strongly suggest that a slow chemical reaction proceeds between quinone and proteins, especially as Benedicenti [1897] found that formaldehyde, which reacts chemically with proteins, is gradually removed by the proteins from aqueous solutions for several weeks, until finally a maximum uptake is indicated.

Some washed gelatin and dialysed egg-albumin were next added to aqueous solutions of quinone and when all the quinone had disappeared, the solutions were extracted with ether. It was possible in each case to detect quinol in the ethereal extract. This suggested that quinone was behaving as a peroxide and that substances were formed in the interaction of proteins and the quinone analogous in constitution to the anilino-derivatives in the case of ethylglycine. It does not exclude, however, the possibility of the alternative reaction in which quinone behaves as a diketone as formerly suggested [Cooper, 1913]. The results show conclusively that quinone reacts with proteins, and that the interaction is of some complexity.

II. The Bactericidal Action of the Quinones.

The remarkably high bactericidal power of quinone has already been referred to in previous communications [Cooper, 1912, 2, and 1913] and the evidence therein presented suggested that this reactivity was associated with a chemical action on certain constituents of the bacterial protoplasm. As benzoquinone exhibits dynamic isomerism and is capable of reacting as either a diketone or a peroxide, it was felt that a further study of this very reactive substance and its homologues from the point of view of their bactericidal power and relation with proteins would be of great interest.

Table IV. *Carbolic acid coefficients.* Temp. 20°.

	B. typhosus		*Staphylococcus py. aur.*	
Disinfectant	15 mins.	30 mins.	15 mins.	30 mins.
p-Benzoquinone	190·0	—	12·6	21·0
,,	80·0	—	10·0	—
p-Toluquinone	20·0	70·0	<4·0	3·25
Xyloquinone	<19·0	<19·0	—	—
Thymoquinone	<19·0	<19·0	—	—
Phenol	1·0	1·1	1·0	1·0
o-Cresol	2·6	—	2·1	—
m-Cresol	2·6	—	2·0	—
p-Cresol	2·6	—	2·4	—
Thymol	25·0	—	—	—
Quinol	1·0	—	—	—
Formaldehyde	0·5	—	0·3	—
Benzaldehyde	10·0	—	10·0	—

For this purpose bactericidal power has been measured in terms of carbolic-coefficients, determined by the method of Chick and Martin [1908] in the absence of organic matter.

It was thought possible that the high germicidal power of the quinones was due to the inhibitory action of traces carried over to the broth-tubes during the process of sub-culturing. To ascertain if this were the case, the organisms were grown in broth at 37° in the presence of definite concentrations of the quinones. The following results were obtained:

Table V.

Benzoquinone concentration	Growth and no growth	Toluquinone concentration	Growth and no growth
1 in 10,000	–	1 in 10,000	–
1 in 100,000	+	1 in 25,000	+
1 in 1,000,000	+	1 in 100,000	+
1 in 10,000,000	+	1 in 1,000,000	+

The results show that no marked inhibitory action took place, and it may be concluded that this factor did not interfere in the carbolic coefficient determinations.

Several matters of interest arise from the results, set out in Table IV. In the first place, it is seen how extraordinarily selective benzoquinone was found to be in its bactericidal action. Not only were there wide differences in the carbolic coefficients determined with distinct organisms, but a considerable divergence also existed when different cultures of the *same* organism were employed. Although selective action is well known in disinfection, the differences observed are not usually so marked as found in the case of benzoquinone. Whether these remarkable differences are due to specific action upon the bacterial protoplasm, or to selective permeability are matters for further investigation. The results also show that the bactericidal power of benzoquinone was enormously greater than that of phenol, and quinol. The bactericidal power of the quinones however diminished as the homologous series was ascended, while in the case of the phenols there was a marked increase. It is thus seen that while toluquinone was still superior to *o*-cresol, the difference was not so great as in the case of the first members of the series. Furthermore, thymoquinone was actually inferior to thymol in germicidal efficiency. Morgan and Cooper [1912] previously found that there was a rise in bactericidal power in ascending an homologous series not only in the case of phenols, but also in the case of the monohydric alcohols and the aliphatic and aromatic amines; heptylamine, for example, possessing the very high carbolic coefficient of 27.

The behaviour of the quinones is thus exceptional and calls for some special explanation.

It is well known that benzoquinone exhibits dynamic isomerism, while the homologous quinones exist mainly in one (peroxide) form. The superiority of benzoquinone in bactericidal efficacy may therefore be due to the continual liberation of its molecules in the nascent reactive condition.

Determinations of the bacterial power of certain aliphatic ketones were next carried out, and the results are tabulated below.

Table VI. *Carbolic acid coefficients.*

Substance	Organism *B. typhosus*	Substance	Organism *Staphylococcus py. aur.*
Benzoquinone	190·0	Quinone	12·60
Acetylacetone	0·20	Acetone	0·07
Formaldehyde	0·50	Diacetyl	0·30
		Formaldehyde	0·30
		iso-Propyl alcohol	0·04

It is seen that the three ketones: acetone, diacetyl, and acetylacetone, are remarkably feeble germicides, and are not only less efficacious than quinone, but even weaker than phenol and formaldehyde.

Acetone, also, is not appreciably stronger than the secondary alcohol, *iso*-propyl alcohol, from which it is derived.

It is also seen that the aliphatic β-diketone acetylacetone which yields a tautomeric "*enolic*" form is much feebler in bactericidal power than benzoquinone which forms a *peroxide* isomeride.

Summary.

1. When proteins are added to solutions of *p*-benzoquinone, the latter slowly disappears, and equilibrium is not attained even after several weeks. Quinol can be detected in the course of the reaction.

2. *p*-Benzoquinone thus appears to react chemically with proteins as a peroxide, and differs fundamentally from the phenols, which are distributed between water and proteins according to the partition-law and behave merely as protein precipitants.

As previously pointed out, *p*-benzoquinone may possibly also react as a ketone with proteins, a simple condensation taking place as in the case of formaldehyde.

3. Benzoquinone possesses a remarkably high bactericidal power, being from 80–190 times as efficacious in destroying *B. typhosus* as quinol and phenol.

4. The bactericidal power of the quinones diminishes as the homologous series is ascended, while with the phenols, alcohols, and amines the opposite is the case. Consequently thymoquinone is less efficacious as a germicide than the corresponding phenol, thymol.

5. The aliphatic ketones, acetone, diacetyl, and acetylacetone are much less efficient as germicides than benzoquinone, or even phenol and formaldehyde.

6. Acetylacetone exists in two tautomeric forms, ketonic and enolic, and it is thus seen that a diketone yielding an enolic form is much feebler in bactericidal efficacy than one, such as benzoquinone, giving a peroxide form.

Conclusion.

Further investigations must be carried out before the mechanism of the bactericidal action of benzoquinone is fully understood, but so far the results show that the extraordinarily high bactericidal power of this substance is associated with its property of exhibiting dynamic isomerism and is probably due to the chemical interaction of the *nascent* peroxide molecules with certain protein-constituents of the bacterial protoplasm.

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