I. THE CHEMICAL ACTION OF QUINONES ON PROTEINS AND AMINO-ACIDS. PART II.

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It was concluded from previous experimental work [Cooper and Haines, 1928, 1] that the bactericidal action of the quinones was associated with their chemical action on the simpler cell-constituents, such as the amino-acids, rather than with their chemical reactivity with the complex colloids, *e.g.* proteins. Subsequently, however, it was found [Cooper and Haines, 1928, 2] that the temperature-coefficient for the reaction between benzoquinone and glycine was the normal one for a chemical process, yet quinone was twenty times more active as a germicide at 37° than at 20°. It was then found however that this considerable accession in bactericidal power with rise in temperature was not restricted to quinone, but was a common property of oxidising disinfectants. It would appear thus that the main factor in the germicidal action of the quinones is the interaction with the amino-acids, but that another factor fundamental in the action of oxidising disinfectants is operating simultaneously.

The earlier work had shown that there was a general correspondence in the relative germicidal power of benzoquinone and toluquinone and their chemical activity with various amino-acids. Benzoquinone was found to react from four to five times as rapidly with amino-acids as toluquinone, while the ratio of their bactericidal action varied on an average from five to seven (and sometimes higher). It was also found that the presence of salts increased the velocity of reaction with glycine. Further experiments have therefore been carried out to ascertain the relative effect of salts on reaction-velocity in the case of both benzo- and tolu-quinone, as, if it could be shown that the inorganic constituents of living cells increased the reactivity of benzoquinone more than that of toluquinone, some explanation of the foregoing discrepancy would be offered.

0.2 % solutions of the two quinones were prepared, (1) in water, and (2) in Ringer's solution (the bicarbonate being excluded). 0.5 g. of glycine was then dissolved in 100 cc. of each solution, and the amount remaining in solution at stated times was determined by withdrawing 5 cc. and titrating by Valeur's method (Table I).

Table I. % remaining in solution at 20°.

(*Benzoquinone.*)

Time min.)	(1) Water	Time (min.)	(2) Ringer's solution
0	100	0	100
10	98.7	10	93.7
30	96.6	35	90.8
70	90.4	100	86.0
120	87.2	160	81.2
235	83.5	240	74.9
360	74.5	360	65.2

(*Toluquinone.*)

0	100	0	100
20	98.2	10	99.5
60	96.4	35	96.4
120	95.2	60	94.8
180	92.3	165	92.1
240	91.1	240	90.5

The saline constituents of Ringer's solution thus considerably accelerate the reaction between benzoquinone and glycine, but have only a slight effect with toluquinone. The inorganic salts present in the cell may thus be supposed to raise the reaction-velocity of benzoquinone, and the great difference in the germicidal power of benzo- and tolu-quinone is explicable.

Apart, however, from velocity considerations, it will be shown that benzoquinone is capable of effecting more cell-destruction than toluquinone per gram-molecule.

Ville and Astre [1895] and Suchanek [1914] showed that the interaction of quinones and amino-compounds took place in several stages as follows:

The addition product can be isolated from either benzene or ether solution; in water or alcohol, however, the reaction continues by oxidation to the formation of the substituted quinone and quinol.

In the case of benzoquinone a dianilido-compound is formed, two nitrogen atoms being introduced. With toluquinone, although a similar addition compound is formed intermediately, the final derivative contains only one nitrogen atom. Thus three molecules of benzoquinone react with two of amino-compound, but two molecules of toluquinone react with only one of the amino-substance.

Xyloquinone forms an addition product, but is incapable of yielding a final substituted quinone, the reaction ceasing at the first stage.

Chloroquinones on the other hand [Astre, 1897] add on two molecules of amino-compound, and both nitrogen atoms finally enter the nucleus with evolution of hydrochloric acid. Chloroquinones are thus as effective as benzoquinone in taking the reaction completely to the oxidation stage.

These observations are analogous to those already made in the case of bactericidal action [Cooper and Haines, 1928, 1]. The homologues are less active as germicides than benzoquinone itself, whilst *m*-dichloroquinone is as efficacious as the latter.

Furthermore, alcohol facilitates the completion of the reaction to the oxidation stage, and also increases the germicidal power of the quinones.

The fact that three molecules of benzoquinone react with two of amino-acid, whilst two molecules of toluquinone are required for the reaction with one molecule of the amino-acid, may also explain the observation already referred to that the difference in the germicidal powers of benzo- and toluquinone is somewhat greater than would be expected from their reaction-velocities with amino-acids. It is evident that per gram-molecule benzoquinone would have a greater destructive effect on the cell than toluquinone in the proportion 3/2.

In order to throw further light on this problem the additive and final oxidation products were prepared from benzoquinone and anthranilic acid, and their germicidal action was studied. The additive product was only germicidal in a concentration as high as 1 in 3000 towards *B. coli* (30 min. at 20°); under the same conditions benzoquinone is active in 1 in 35,000. The oxidation product was too insoluble for examination. Although it would seem that it is the final oxidation process that plays the chief rôle in the bactericidal action of the quinones, the fact that benzoquinone suffers such an extensive depreciation in germicidal power merely by forming an addition compound with anthranilic acid suggests that the bactericidal process is also bound up to a certain extent with the preliminary addition reaction.

Reference has already been made to the finding that the temperature-coefficient for the interaction of benzoquinone and glycine is the normal one for a chemical process, whilst at 37° the quinone is twenty times as powerful a germicide as at 20°.

The exact germicidal temperature-coefficient, however, had not been determined under the conditions employed, all that had been ascertained being the relative concentration required for disinfection in a fixed period at 20° and 37°.

The temperature-coefficient for bactericidal action was therefore next measured by means of a modified technique.

Selected concentrations of phenol and quinone were inoculated with a definite volume of culture at 20° and 37°, and by subculturing at stated intervals into nutrient broth the periods required for complete disinfection at the different temperatures could be ascertained. The ratio of the times is a measure of the temperature-coefficient of the process (Table II).

Table II.

	Period of disinfection (min.)		Temperature-coefficient per 10°
	20°	37°	
<i>B. coli</i>			
Phenol, 1 in 200	150	27.5	3.2
Quinone, 1 in 50,000	72.5	7.5	5.7
<i>B. fluorescens</i>			
Phenol, 1 in 200	67.5	12.5	2.9
Quinone, 1 in 80,000	42.5	< 4	> 6

The observations confirm the original suppositions; the temperature-coefficient of disinfection in the case of quinone is 6 or even higher, the coefficient for the chemical action on glycine being the normal one (2 for a 10° rise). In the case of phenol the temperature-coefficient for germicidal action is lower (approximately 3) and agrees more closely with the coefficient for the process of protein-coagulation by phenol, viz. 2 [Cooper and Haines, 1928, 2].

m-Cresol, on the other hand, was found not to increase in germicidal power with rise in temperature, 1 in 500 concentration being equally bactericidal at 20° or 37°. It was of interest therefore to extend the protein coagulation experiments to the case of this homologue of phenol.

100 cc. of egg-white were made up to 200 cc. with distilled water and filtered; the filtrate was diluted with an equal volume of water and again filtered. 100 cc. of the final filtrate were mixed with 100 cc. of 1 % *m*-cresol solution in a large bottle and kept at constant temperature in a thermostat. The rate of coagulation was determined at 20° and 30°, 10 cc. of the mixture being withdrawn at intervals and rapidly filtered.

The amount of protein in the filtrates was then estimated by heat-coagulation and weighing (Fig. 1).

The results, plotted in Fig. 1, show that the velocity of coagulation by *m*-cresol is only very slightly increased by rise in temperature, and are thus consistent with the bactericidal observations. The physico-chemical explanation of the germicidal power of the phenols is thus quite in accordance with the facts.

In the case of quinone, however, the chemical interpretation already put forward, whilst entirely consistent with many observations, does not at the present time account for the high temperature-coefficient for germicidal action.

Table III. % quinone taken up from the solution.

Pathological source of urine	Benzoquinone		Toluquinone		Ratio	
	$\frac{1}{2}$ hr.	1 hr.	$\frac{1}{2}$ hr.	1 hr.	$\frac{1}{2}$ hr.	1 hr.
Leucaemia	7.7	18.6	3.6	8.4	2.1	2.2
"	67.6	—	37.9	—	1.8	—
Nephritis	11.2	25.0	8.2	18.1	1.4	1.4
Jaundice	5.44	25.0	5.2	25.1	1.0	1.0
Haematuria	41.0	66.0	28.6	53.1	1.4	1.3
"	58.4	—	40.6	—	1.4	—
Glycosuria	35.8	—	6.1	—	5.9	—
"	6.8	—	1.3	—	5.2	—

Fig. 1. Velocity of coagulation of albumin by *m*-cresol.

The possible application of the quinones in medicine.

It was previously shown [Cooper and Haines, 1928, 1] that alcoholic solutions of benzoquinone react rapidly with amino-acids, and since the nitrogenous constituents of urine vary considerably in health and disease it was thought that the amount of reaction might be of use as an index in diagnosis. Accordingly experimental work in this direction has been carried out. The clinical significance of the benzoquinone/toluquinone reactivity ratio has also been considered. In general, 10 cc. of the specimen of urine were allowed to react with an equal volume of 0.4 % solution of the quinones in 25 % alcohol for definite periods, the course of the reaction being followed by the volumetric estimation of the quinone remaining unused in the solution (Table III).

The chemical reactivity ratio of the two quinones is thus highest in the case of glycosuria (5.5), intermediate in value with leucaemia (2), and lowest in nephritis, jaundice and haematuria (1.4). It would thus seem that the deter-

mination of this ratio would afford a basis for a test which might be of value in medical diagnosis. The process resolves itself into a simple titration with thiosulphate under standard conditions, and can be carried out in 30 minutes to 1 hour.

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