THE INFLUENCE OF TEMPERATURE ON BACTERICIDAL ACTION.

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(With 1 Figure.)

Bactericidal action has in general been found to be increased by rise in temperature. Thus, Chick and Martin (1908a) worked out the cases of phenol, mercuric chloride, and silver nitrate very fully, and found that germicidal power usually increased about three-fold for a rise in temperature of 10° C. (although higher coefficients of 7 or 8 were sometimes observed with phenol). Cooper and Mason (1928), however, found that in the case of hydrazine hydrate and picric acid (with *B. fluorescens*) germicidal power was unaffected by rise in temperature. This exceptional behaviour complicated the natural division of disinfectants into "chemical" and "physico-chemical" germicides, evidence for which had been previously obtained by a study of the selective action of various germicides on *Bacillus pyocyaneus* and related organisms (Cooper and Mason, 1928). The effect of temperature on bactericidal action has therefore been still more widely studied, and the results obtained are recorded in the present paper.

The Chick-Martin method (1908b) was employed in this work for the estimation of bactericidal power. The organisms used were *B. coli communis* and *B. fluorescens non-liquefaciens*, and the period of disinfection was 30 minutes at 20° and 37° C.

Inspection of the foregoing results shows that with *B. coli* the compounds examined fall into three groups:

1. Those showing no measurable change in bactericidal activity from 20–37° C. (It is not permissible to work above this temperature, as "heat-disinfection" would then become predominant.)
2. Those showing a change of from two to three times.
3. Those showing a much greater change of from eight to twenty times.

With *B. fluorescens non-liquefaciens*, on the other hand, nearly all the compounds examined show only a relatively small change in germicidal action with rise in temperature.

It is remarkable that with *B. coli* the substances showing little or no change all belong to the "physico-chemical" class of germicides, and that most of them have at the same time reducing properties, comparable to the results obtained by Cooper and Mason (1927). Those giving high ratios with

Table I. *Chick-Martin test, Half hour period. Germicidal concentrations.*

	B. coli		*B. fluorescens non-liquefaciens*	
	20° C.	37° C.	20° C.	37° C.
1.				
Hydroxylamine hydrochloride	1 in 40	1 in 40		
Pyrogallol	150	150		
m-cresol	500	500		
Formaldehyde	1000	1000		
p-bromphenol	1250	1250		
2.				
Phenol	1 in 120	1 in 220	1 in 150	1 in 400
Hydrogen peroxide	700	1100	1300	2000
Ethyl alcohol	3·5	8	5	12
Acetone	3·5	9·5	5·5	8·5
Mesityl oxide	75	125	—	—
Resorcinol	1 in 40	1 in 75	1 in 75	1 in 150
Quinol	1 in 400	1 in 1500	1 in 3500	1 in 8500
Formaldehyde	—	—	400	1300
Picric acid	—	—	2300	2800
Hydroxylamine hydrochloride	—	—	400	600
Pyrogallol	—	—	450	550
Potassium permanganate	—	—	20,000	40,000
3.				
Picric acid	1 in 400	1 in 3400		
Benzoquinone	35,000	300,000	1 in 60,000	1 in 300,000
Toluquinone	7000	175,000	8500	40,000
Quinhydrone	12,500	125,000		
2–6 dichlor-quinone	40,000	250,000		
Potassium permanganate	7500	50,000		

rise in temperature are mostly "chemical" germicides, and, moreover, oxidising agents.

The observations recorded in the previous papers (*loc. cit.* 1927, 1928) showed that the "physico-chemical" germicides, *i.e.* those substances exerting a precipitating or denaturating action on proteins, were powerfully bactericidal towards *B. fluorescens*, whilst the "chemical" disinfectants, the germicidal action of which was associated with their chemical reactivity towards certain of the constituents of protoplasm, attacked *B. coli* more readily. At the same time, *B. fluorescens* being more markedly aerobic than *B. coli* was also more susceptible to reducing agents than *B. coli*, and reducing substances, *e.g.* hydrazine, hydroxylamine, were thus classified with the "physico-chemical" disinfectants. The experimental work upon which this conclusion was based was carried out by means of the inhibitory test. This method, unlike the Chick-Martin test, covers a period of 48 hours at 37° C. and thus measures the efficacy of a compound both in restraining the growth of an organism and in exerting a slow germicidal action.

Examples of the "physico-chemical" germicides are the alcohols, phenols, acetone, hydroxylamine hydrocloride, and "chemical" germicides are represented by quinones, hydrogen peroxide. A survey of the results tabulated in Table I shows that this general principle still holds when the shorter time test

is used either at 20° or 37° C., the "physico-chemical" germicides—alcohol, phenol, pyrogallol, resorcinol, quinol, hydroxylamine hydrochloride—invariably attack *B. fluorescens* more readily than *B. coli* under all experimental conditions employed.

Certain compounds however behave anomalously; picric acid acts as a "physico-chemical" germicide at 20° C., but is a "chemical" disinfectant at 37° C. as also observed by the inhibitory method (*loc. cit.* 1927, 1928).

Hydrogen peroxide although indicated by the inhibitory process as a "chemical" disinfectant behaves as a "physico-chemical" germicide when tested by the Chick-Martin method at 20° or 37° C. The quinones, formaldehyde, and potassium permanganate also vary in character according to the conditions of the experiment, owing to their temperature coefficients being different for *B. coli* and *B. fluorescens*. The explanation for these divergencies is difficult in the present state of our knowledge. A likely interpretation however is that the variable substances may possess the capacity of *both* reacting chemically and also denaturating the cell constituents, and that either process may predominate under particular experimental conditions.

The Velocity of Coagulation of Egg-Albumin with Phenol.

The germicidal power of phenol has been ascribed to its coagulating or physico-chemical action on the cell proteins, as has previously been stated. It is therefore to be expected that the temperature coefficient of coagulation should show some similarity with the increase of germicidal power of this compound with temperature. Over a range of 10° C., the germicidal action of phenol has a temperature coefficient, varying from 2 to 8, depending on the organism used and other experimental conditions. Chick and Martin (1910, 1911) showed that "heat-coagulation" of egg-albumin consisted of two processes, denaturation and agglutination, and that denaturation had the extraordinarily high temperature coefficient of 1·3–1·9 per *degree*, whilst agglutination had the more normal coefficient of 2–2·5 per 10 degrees. Since it by no means follows that heat-coagulation and phenol coagulation obey the same laws, the following experiments were instigated to investigate this question.

The whites of four eggs, giving approximately 100 c.c., were made up to 200 c.c. and filtered. The filtrate was diluted with an additional 200 c.c. of water and again filtered. No attempt has been made to purify the protein in any way, because, although it has been shown that the presence of salts exerts a considerable influence on coagulation, these salts are always present in living organisms, and it is from this standpoint that the experiments were carried out. 100 c.c. of the solution so obtained were mixed with 100 c.c. of 2 per cent. phenol solution in a large bottle fixed in a thermostat and fitted with a mechanical stirrer. 10 c.c. were withdrawn immediately on mixing, and similar quantities at stated intervals, and quickly filtered through filter papers of special quality. The amount of protein in the filtrate was estimated

by coagulating by heating in the presence of ammonium sulphate and a trace of acetic acid.

The coagulum so obtained was filtered off on a Gooch crucible and dried at 110° C.

Table II.

Time (min.)	Weight of protein remaining per 10 c.c. (g.)	Time (min.)	Weight of protein remaining per 10 c.c. (g.)	Time (min.)	Weight of protein remaining per 10 c.c. (g.)
1. 20° C.		2. 30° C.		3. 40° C.	
0	0·0719	0	0·0714	0	0·0714
10	0·0671	20	0·0653	20	0·0619
30	0·0656	35	0·0614	50	—
50	0·0640	50	0·0600	90	0·0536
90	0·0620	90	0·0576	150	0·0532
125	0·0610	150	0·0560	260	0·0500
180	0·0585	240	0·0536	300	0·0490
240	0·0566	300	0·0532		
300	0·0564				

These results are shown graphically in the accompanying curves (Fig. 1). The equation to the curve is unknown, and the velocity constant cannot therefore be calculated directly, but a comparison of the velocities at different

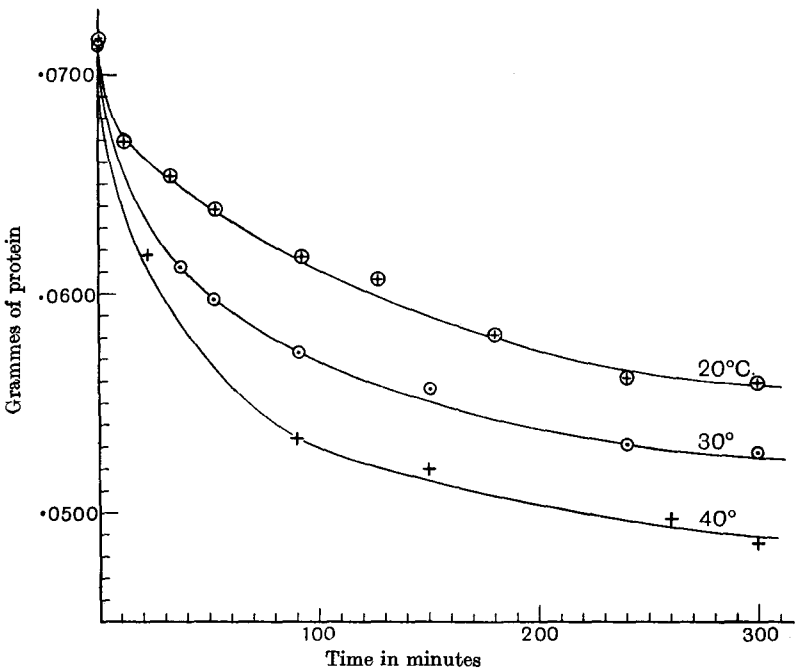

Fig. 1.

temperatures can be made and the temperature coefficient thus obtained by comparing the times taken to pass from one concentration of albumin to another on each curve.

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		Coefficients for 10° C.		
		20°/30°	20°/40°	30°/40°
(i) 0·0650–0·0600				
20°	130 – 38 = 92			
30°	56 – 18 = 38	2·4	2·5	2·1
40°	30 – 12 = 18			
(ii) 0·0675–0·0575				
20°	214 – 14 = 200			
30°	98 – 8 = 90	2·3	2·2	2·0
40°	50 – 5 = 45			
(iii) 0·0650–0·0575				
20°	214 – 38 = 176			
30°	98 – 18 = 80	2·2	2·2	2·1
40°	50 – 12 = 38			

Mean value 2·2 *per* 10° C. *rise in temperature.*

There is thus on the whole a very close parallel between the influence of temperature on the germicidal action of phenol and on its protein coagulating power, quite sufficient, in fact, to substantiate the previous conclusion that phenol owes its bactericidal efficacy to this physico-chemical action, although no explanation is given for the abnormally high temperature coefficients sometimes observed in phenol disinfection.

VELOCITY OF REACTION OF QUINONES WITH GLYCINE.

It has been suggested that there is some factor common to all the substances of Group 3 in Table I, such as their oxidising properties, in some way responsible for the high temperature coefficient of their germicidal action. In order to test this to some extent a series of velocity experiments have been carried out with quinones and glycine, as it has been previously put forward (Cooper and Haines, 1928) that the quinones owe their germicidal action to their great reactivity with simple amino-acids.

In general 0·5 g. of glycine was allowed to react with 100 c.c. of 0·2 per cent. quinone in a stoppered bottle at the stated temperature, and the amount of quinone used up estimated by titrating the excess by the method of Valeur.

The results are set out in Table III.

Table III.

0·5 g. glycine + 100 c.c. 0·2 % quinone. Amount of quinone remaining in terms of standard thiosulphate.

Time (min.)	20° (c.c.)	37°	*K*20	*K*37
0	19·90	19·90	·00002297	·00002685
30	17·90	17·4	1985	3945
60	16·70	13·8	2322	5245
120	13·60	8·6	1686	4963
180	13·20	6·6	1644	5890
240	11·90	4·2	1713	5747
300	10·50	3·3	1654	5697
360	9·70	2·6	1723	5977
420	8·60	1·9	1909	5578
480	7·3	1·7		
∞	2·4	0·50		

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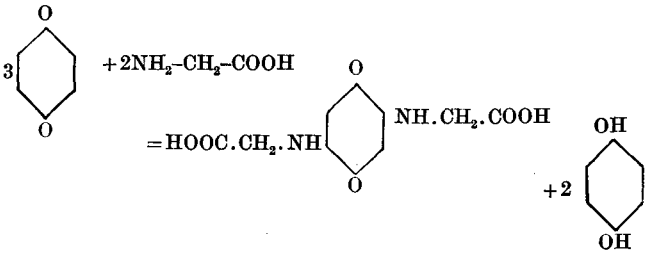
The velocity constant has been calculated assuming the reaction of Fischer and Schrader (*Ber.* 1910, **43**, 525) to be bimolecular.



A consideration of the results shows that K is not constant at the commencement of the reaction, but becomes steady in the later stages. The average value for the temperature coefficient for a 10° rise in temperature is 2·02. The initial variation in the value of the coefficient probably means that the reaction is not solely of the type indicated in the foregoing equation. A second set of results was obtained, and the temperature coefficient in this case derived from the curve, as in the experiments on velocity of protein-coagulation. These results are set forth in Table IV, the experimental conditions being the same as before (Table III).

Table IV.

Time (min.)	37° (c.c.)	20° (c.c.)
0	21·7	21·7
30	20·00	21·25
60	18·0	20·60
120	14·9	19·80
180	11·9	18·20
240	—	15·9
420	5·8	14·85
480	5·0	14·0
520	4·35	13·3
24 hrs	1·05	7·05

Temperature coefficient = 2·3 per 10° rise in temperature.

The temperature coefficient of this reaction is thus the normal one for chemical processes, and, although considerable evidence has been brought forward in support of the view that the germicidal action of the quinones is associated with their chemical reactivity towards the simple cell-constituents, *e.g.* amino-acids, this explanation does not therefore account for the fact that the bactericidal power of the quinones is so abnormally increased by rise in temperature (Table I).

It has been previously shown (Cooper and Haines, 1928), however, that the presence of certain substances, such as alcohol and the salts constituting Ringer's solution, accelerated the reaction between quinone and glycine. In view of these results, it was possible that this reaction would be modified or accelerated by the conditions obtaining within the living cell, and further experimental work concerning the influence of mineral salts on velocity of reaction was therefore put in hand. The results obtained are given in Table V,

and expressed in terms of the percentage of quinone still remaining in solution at the end of definite times.

Table V.

1. Benzoquinone and glycine at 20° C.

(i) In water

Time (min.)	% of quinone remaining
0	100
10	98·68
30	96·6
70	90·4
120	87·2
235	83·5
360	74·5

(ii) In Ringer's solution

Time (min.)	% of quinone remaining
0	100
10	93·7
35	90·8
100	85·98
160	81·2
240	74·9
360	65·2

(iii) In water + 0·05 g. $MgHPO_4$

0	100
10	98·2
35	93·9
95	83·3
130	78·5
230	72·6
345	66·2

(iv) In Ringer's solution + $MgHPO_4$

0	100
15	96·5
30	86·9
60	81·8
120	73·2
180	65·6

(v) In water + 0·05 g. manganese chloride

0	100
15	97·3
30	95·2
60	93·1
120	87·3
180	82·0

(vi) Control with benzoquinone in Ringer's solution + $MgHPO_4$. (*No* glycine)

0	100
15	100
40	99·0
170	96·3
250	96·3
295	96·2

(vii) In Ringer's solution + $MgSO_4$

0	100
20	97·4
60	95·8
120	91·3
180	88·0
240	82·0

(viii) In Ringer's solution + manganese phosphate

0	100
20	98·4
60	95·8
120	90·0
180	85·5
240	79·6

2. Toluquinone and glycine

(i) In water

0	100
20	98·2
60	96·4
120	95·2
180	92·3
240	91·1

(ii) In Ringer's solution + $MgHPO_4$

0	100
20	99·5
30	99·5
60	91·3
120	86·5
180	82·2
240	77·8

(iii) Influence of temperature: Benzoquinone and glycine

20°

In 25 % alcohol

0	100
30	91·0
60	83·7
120	72·9
180	64·2
300	50·3

In Ringer's solution + $MgHPO_4$ + $MnCl_2$

0	100
30	88·9
60	85·8
120	76·7
180	74·6
300	65·0

37°

In 25 % alcohol

0	100
30	83·5
90	55·2
150	37·8
210	—
270	18·9

In Ringer's solution + $MgHPO_4$ + $MnCl_2$

0	100
30	90·6
90	72·3
150	60·6
—	—
270	38·4

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A survey of the tabulated results indicates that the presence of alcohol, and certain mineral substances, such as magnesium and manganese salts, considerably accelerates the reaction between quinones and glycine. For certain time periods the increase in velocity may be as much as from two to five-fold. These observations suggest the interesting point that the quinones may be activated in germicidal power in consequence of the presence of certain salts within the living organisms. The temperature coefficient, however, for the chemical reaction between benzoquinone and glycine is not increased by the presence of salts, or alcohol, and thus an explanation of the high temperature coefficient for the germicidal action of the quinones is still wanting. Since, however, the germicidal power of other compounds, as well as the quinones, *e.g.* picric acid and potassium permanganate, increases abnormally fast with rise in temperature, it may still hold that the bactericidal action of the quinones is due to their chemical reactivity with the simpler cell-constituents, and that there is some other additional factor common to all the foregoing disinfectants associated with their high temperature coefficient.

The results bring out the interesting point in practical disinfection that such disinfectants as picric acid and permanganate are far superior for use at 37° C. than phenol, cresol or formaldehyde, although the differences are much less marked at ordinary room temperature.

SUMMARY.

1. The influence of temperature on the germicidal power of selected disinfectants has been studied, and it has been found that germicides may be classified broadly into three main groups, viz.

Germicidal power (*a*) unaffected by rise in temperature: chiefly chemical substances, possessing reducing properties;

(*b*) approximately doubled by rise in temperature from 20 to 37° C.—phenol, alcohol;

(*c*) increased as much as 10 or 20-fold by the same temperature rise: oxidising agents.

2. The effect of temperature on bactericidal action is different for *B. coli* and *B. fluorescens*, thus causing very complex changes in selective action, which are discussed in this paper.

3. The temperature coefficient for the process of protein-precipitation by phenol is 2·2 per 10° C. rise in temperature, and thus similar in magnitude to the coefficient for disinfection by phenol. This supports the view that the germicidal action of phenol is associated with its denaturating effect on the cell-proteins.

4. Previous experimental work had suggested that the bactericidal action of the quinones was due to their chemical reactivity with the simple cell-constituents, *e.g.* the amino-acids, rather than with the complex colloids. The temperature coefficient of the reaction between benzoquinone and glycine is, however, the normal one, viz. 2 for 10° C. rise, for chemical action, although

the germicidal action of the quinones may be actually 20 times more effective at 37° than at 20° C. The presence of certain inorganic cell-constituents accelerates the velocity of reaction between quinone and glycine, but does not increase the temperature coefficient.

5. The bactericidal power of picric acid and potassium permanganate, as well as the quinones, is greatly increased by rise in temperature: and there would appear to be some other factor common to all these disinfectants associated with their high temperature coefficient.

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