The isolation of the L type of growth was made possible both in the case of *flavobacterium* and of *funduliformis* by the elimination of viable bacteria by autolysis. The other cultures also in which the L type of colonies were observed, as in certain strains of *Bacillus coli, Bacillus influenzae* and of the gonococcus, possess a tendency to autolyze. In these cultures, however, the autolyzing was not complete and thus far all attempts to isolate the L type of growth have remained unsuccessful because the transplants were overgrown by bacteria.

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Relative Germicidal Action of Some Halogenated Phenols and Their Phenolates.

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Klarmann, Shternov and von Wowern[1] showed that the substitution of one, 2, and 3 atoms of chlorine into the phenol molecule led to a progressive increase in the germicidal action of the resulting compounds. Unfortunately, at the same time the solubility of the phenolic compounds decreased markedly with substitution. The sodium salts of these halogenated phenols possess a considerably greater solubility than the phenols themselves, but there is no explicit information available in the literature as to the actual germicidal power of the phenolates.

Tilley and Schaeffer[2] showed that the germicidal efficiency of phenol decreased with an increase in percentage of sodium hydroxide added until the percentage of sodium hydroxide equalled or slightly exceeded that required to neutralize the phenol. Phenol is a weak acid with the acid dissociation constant $K'_a = 1.06 \times 10^{-10}$ at 25°C.[3] Consequently a completely neutralized solution of phenol will be strongly alkaline, and an attempt to evaluate the actual germicidal action of the phenolate is complicated by the alkalinity of the solution. The substitution of chlorine atoms into the phenol molecule increases

1 Klarmann, E., Shternov, V. A., and von Wowern, J., *J. Bact.*, 1929, **17**, 423.

2 Tilley, F. W., Schaeffer, J. M., *J. Agr. Res.*, 1931, **43**, 611.

3 *International Critical Tables*, prepared by National Research Council of the U.S.A., 1929.

the strength of the molecule as an acid and makes it possible to evaluate the relative germicidal action of some undissociated phenols and their phenolates without undue interference by excess acidity or alkalinity.

In this investigation 3 substituted phenols: o-chlorophenol, 2, 4 dichlorophenol and 2, 4, 6 trichlorophenol were tested in moderately acid and alkaline solutions. The acid series of solutions were made by diluting weighed quantities of the several phenols with M/60 phosphate buffer of approximately pH 6.1. The alkaline solutions were made by adding standard sodium hydroxide to weighed samples of the phenols to adjust them to the neighborhood of pH 9.8 and diluting with M/60 carbonate buffer at pH 9.8. The pH of each solution tested was accurately determined with a glass electrode.

Germicidal tests were made using the Food and Drug Administration[4] procedure for *Staphylococcus aureus,* except that the temperature of the experiment was 25°C and that subcultures were made into molten agar at 42-43°C and plates poured instead of using tubes of broth. Colony counts were made after 48 hours' incubation at 37°C.

The relative germicidal effects in acid and alkaline solutions are shown in Table I.

TABLE I.
Germicidal Action of Chloro-Substituted Phenols in Acid and Alkaline Solutions.

Compound	Conc. of phenol in g per L	pH of sol.	Colonies per plate		
			5 min	10 min	15 min
o-chlorophenol	4.00	6.09	0	0	0
	3.63	6.10	24	0	0
	3.33	6.10	320	37	4
	58.8	9.93	0	0	0
	39.2	9.89	20	0	0
	29.4	9.87	1100	670	380
2,4-dichlorophenol	0.833	6.10	3	0	0
	0.769	6.10	39	0	0
	0.715	6.10	344	25	0
	40.7	9.98	0	0	0
	30.5	9.93	12	0	0
	24.4	9.90	124	36	0
	16.3	9.86	1300	700	370
2,4,6-trichlorophenol	.571	5.84	0	0	0
	.500	5.86	45	0	0
	.444	5.88	450	58	6
	40.0	9.76	0	0	0
	34.3	9.80	14	3	0
	30.0	9.82	230	40	0

[4] Circular No. 198, 1931, U. S. D. A.

TABLE II.
Composition of Critical* Germicidal Solutions.

Compound	pK'_a at 25°C	pH of sol. tested	Total phenol added in moles/L $\times 10^3$	Conc. of undissociated phenol in moles/L $\times 10^3$	Conc. of phenolate ion in moles/L $\times 10^3$
o-chlorophenol	8.5	6.10	28.2	28.1	.1
		9.89	305.	11.9	293.1
2,4-dichlorophenol	7.7	6.10	4.72	4.62	.1
		9.93	187.3	1.2	186.1
2,4,6-trichlorophenol	6.2	5.86	2.53	1.68	.85
		9.80	173.6	.04	173.6

*Solutions most nearly sterilizing in ten minutes but not in five at 25°C.

It is evident from Table I that there is a sharp drop in the germicidal activity when the solutions are made alkaline.

For purposes of comparison the solutions most nearly sterilizing in ten minutes but not in five were designated as critical solutions. The actual concentrations of undissociated phenols and of phenolates were calculated for these solutions from the total concentration of the phenol and from the pK'_a values of the substituted phenols. The pK'_a values for 2,4-dichlorophenol and 2,4,6-trichlorophenol were obtained from Krahl and Clowes[5] while that for o-chlorophenol was obtained from Murray and Gordon.[6] The results are expressed in Table II.

It is evident from Table II that the germicidal strength of the phenols increases directly with the number of chlorine atoms substituted, thus confirming the results of Klarmann, Shternov and von Wowern.[1] Comparison of the acid and alkaline solutions show that the undissociated phenolic compounds are far more germicidal than their phenolates, although the phenolates are not without toxicity. In the case of 2,4,6-trichlorophenol it is possible to get an estimate of the relative germicidal strength of the undissociated compound as compared with the phenolate since only a trace of the undissociated phenol is to be found in the solution at pH 9.80. From the ratio of the concentration of phenolate at pH 9.80 to that of the phenol at pH 5.86 it appears that the undissociated phenol is approximately 100 times more active than its phenolate under the conditions of the experiment.

[5] Krahl, M. E., and Clowes, G. H. A., *J. Cell. and Comp. Physiol.*, 1938, **11**, 1.
[6] Murray, J. W., and Gordon, N. E., *J. Am. Chem. Soc.*, 1935, **57**, 110.