CXXV. STUDIES ON SELECTIVE BACTERICIDAL ACTION.

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CASES of selective bactericidal action have been frequently observed by different investigators.

In the course of a comprehensive research on the germicidal power of nitroso-compounds, the authors have, however, noticed a very uncommon type of selective action. From the results obtained it appears that *Bacillus pyocyaneus* is abnormally sensitive to the action of the so-called physico-chemical class of germicides, *e.g.* phenol, alcohol, whilst it is very resistant to the action of germicides that owe their efficacy to definite chemical action upon the protoplasmic constituents of the organism, *e.g.* nitroso-compounds, salts of heavy metals, etc. As the results may contribute to our knowledge of the chemical constitution and colloidal structure of micro-organisms, and further work on these lines would seem to offer a promising field for research, the main results of the investigation are summarised and discussed in the present paper.

The method employed for estimating germicidal power may be briefly described as follows.

A culture of the particular micro-organism used was incubated for a definite period in the presence of various concentrations of the germicides under examination, and the effect on the growth of the organism was observed. The exact technique consisted in first adding to definite volumes of leuco-peptone broth (8 cc.) varying quantities of sterile distilled water, in such amount that on subsequently adding the desired volume of disinfectant solution, the total volume would be 10 cc. Before the addition of the germicide, however, two drops of a 24 hours' culture of the organism were added to each tube. After well shaking, the disinfectant was then introduced. After further mixing the tubes were incubated at 37° for two days, at the end of which the tubes were examined. In this way it was possible to determine the exact concentration which was just sufficient to prevent the growth of the organism. When several compounds are being compared by this method, it is necessary to test them simultaneously, to exclude errors due to variations in the organisms. Although it would appear that inhibitory action is being determined by this method, yet there is evidence that we are in reality measuring germi-

cidal power, since by sub-culturing blank tubes into fresh broth, no growths occurred in the sub-culture tubes. Different varieties of the organisms were used in order to exclude possible errors due to idiosyncrasy of any one culture. It is of importance to point out that the selective action of the physico-chemical group of germicides upon *B. pyocyaneus* is not necessarily shown in short period experiments, but the 48 hours' test used in this investigation has been found to be fully adequate for the purpose of differentiation.

The marked germicidal action of *p*-nitrosophenol and *p*-nitrosoaniline was first pointed out by Cooper and Woodhouse [1923], and in the present communication a large number of nitroso-compounds—some of quite a different type—have been examined.

The bactericidal powers of these substances are tabulated in Table I, and from a consideration of the results, the following conclusions may be drawn.

Table I. *Germicidal Concentrations of Nitroso-compounds.*

Substance	<i>B. coli communis</i> *	<i>B. typhosus</i>	<i>B. pyocyaneus</i>
Phenol	1 in 550	1 in 680	1 in 870
<i>p</i> -Nitrosophenol	1 " 18,000	—	1 " 2,000
<i>p</i> -Nitroso- <i>o</i> -cresol	1 " 16,500	—	1 " 1,200
<i>p</i> -Nitroso- <i>m</i> -cresol	1 " 16,500	—	—
<i>p</i> -Nitrosoresorcinol	1 " 2,100	—	—
Dinitrosoresorcinol	1 " 2,100	—	—
α -Nitroso- β -naphthol	1 " 45,000	—	—
<i>p</i> -Nitrosoaniline	1 " 120,000	1 in 200,000	1 in 33,000
<i>p</i> -Nitroso-methyl-aniline	1 " 140,000	1 " 270,000	1 " 11,000
<i>p</i> -Nitroso-ethyl-aniline	1 " 70,000	1 " 160,000	1 " 3,500
<i>p</i> -Nitroso-di-methyl-aniline	1 " 170,000	1 " 300,000	1 " 8,500
<i>p</i> -Nitroso-di-ethyl-aniline	1 " 50,000	1 " 125,000	1 " 1,400
<i>p</i> -Nitroso- <i>o</i> -toluidine	1 " 200,000	—	1 " 18,000
<i>p</i> -Nitroso-phenyl-methyl-nitrosamine	1 " 18,000	1 " 62,000	>1 " 3,000
<i>p</i> -Nitroso-phenyl-ethyl-nitrosamine	1 " 13,000	1 " 140,000	>1 " 3,000

* The culture used was Houston II. Results of much the same order were obtained with Esherich.

All the nitroso-derivatives tested are active germicides, nitrosoaniline and its homologues being more efficacious than the nitrosophenols, and the di-nitroso-compounds.

The nitrosophenols are remarkable insomuch as the introduction of a methyl group results in a diminution in germicidal power, whilst in the case of the phenols it is well known that the cresols are much superior to phenol itself.

Nitroso- and dinitroso-resorcinol are less active than the monohydric nitrosophenols, while α -nitroso- β -naphthol is the most powerful of all the phenolic derivatives examined.

The homologous series of nitrosoanilines behave in a very peculiar manner. With the coliform organisms, the introduction of *methyl* groups slightly increases bactericidal power, whilst that of *ethyl* groups brings about an appreciable fall in efficacy. Nitroso-di-methyl-aniline is therefore a more active germicide than the isomeric nitroso-ethyl-aniline. Symmetrical substitution is thus more favourable than the dissymmetrical type, as has also been observed in the case of tellurium compounds [Morgan, Cooper and Burt, 1924].

The results with *B. pyocyaneus* are different from those observed with *B. coli* and *B. typhosus*.

B. pyocyaneus is consistently more resistant than the latter organisms to the action of all the nitroso-compounds of the different types examined, while, on the other hand, it shows a specific susceptibility to the germicidal action of phenol itself. In both the nitroso-phenol and aniline series the introduction of alkyl groups also causes a marked diminution in bactericidal power in the case of *B. pyocyaneus*.

It is interesting, furthermore, to note that the substitution of a nitroso group in the secondary amine radicles of nitroso-methyl- and nitroso-ethyl-aniline, forming nitroso-nitrosamines leads to a collapse in germicidal action.

The anomalous behaviour of *B. pyocyaneus* referred to above suggests that this organism is abnormally susceptible to a germicide of the physico-chemical or colloid-precipitating type, *e.g.* phenol, whilst towards typical chemical germicides, such as nitroso-compounds, which readily condense with nitrogenous substances, it is extremely resistant. It was evident, however, that before any general conclusion could be reached, it would be necessary to test a large number of compounds of widely different chemical nature, and this work was therefore next undertaken and the results are tabulated in Table II.

A consideration of the above results shows that the compounds which are known to react chemically with the constituents of protoplasm and may therefore be classified as chemical germicides, *e.g.* nitroso-compounds, aldehydes, benzoquinone, hydroxylamine, salts of heavy metals (mercury and cadmium) are with one exception much more efficacious against *B. coli* than against *B. pyocyaneus*. The only exception is formaldehyde (and occasionally hydroxylamine), which attacks the two organisms almost with equal readiness. The tellurium- β -diketone which on account of its activity in extreme dilution is in all probability a chemical germicide, also exerts a selective action on *B. coli*. The results obtained with cadmium sulphate are of interest as the germicidal power of the salts of this metal has not apparently been examined previously. It is seen that cadmium sulphate is quite efficacious as a germicide, but is less active than mercuric chloride.

The results with the physico-chemical germicides are more complex, but quite significant, when the interfering factors are considered. It is seen in the first place that the homologous series of aliphatic alcohols, also benzyl alcohol, phenol, chloro-phenols, quinol, and toluquinol, differ from the above chemical germicides in preferentially attacking *B. pyocyaneus*. Thus, quinol is nearly four times as efficacious against *B. pyocyaneus* as against *B. coli*. These results show quite clearly that *B. pyocyaneus* is abnormally susceptible to the action of the physico-chemical group of germicides.

There are, however, some anomalous results that call for explanation. It has already been shown that the introduction of an alkyl group into the benzene

nucleus or amino-group of nitroso-compounds has the unusual effect of greatly diminishing the bactericidal action of nitroso-compounds against *B. pyo-*

Table II. *Differentiation between B. pyocyaneus and B. coli.*

		Bactericidal concentrations	
<i>The alcohols</i>		<i>B. pyocyaneus</i>	<i>B. coli</i> (Houston II)
Methyl alcohol		1 in 14	1 in 9
Ethyl alcohol		1 " 43	1 " 21
Propyl alcohol		1 " 67	1 " 43
Isopropyl alcohol		1 " 43	1 " 30
Benzyl alcohol		1 " 260	1 " 177
Monochlorohydrin		1 " 60	1 " 60
<i>The phenols</i>			
Phenol	(i)	1 " 920	1 " 650
	(ii)	1 " 780	1 " 540
<i>o</i> -Cresol		1 " 1,215	1 " 1,215
<i>m</i> -Cresol		1 " 1,215	1 " 1,215
<i>p</i> -Cresol		1 " 1,215	1 " 1,215
Thymol	(i)	1 " 1,255	1 " 4,700
	(ii)	1 " 835	1 " 4,300
<i>o</i> -Chlorophenol		1 " 4,850	1 " 2,430
<i>p</i> -Chlorophenol	(i)	1 " 4,160	1 " 4,160
	(ii)	1 " 4,160	1 " 3,500
Picric acid		1 " 290	1 " 600
<i>The quinols</i>			
Quinol		1 " 2,550	1 " 710
Toluquinol		1 " 1,040	1 " 700
<i>p</i> -Xyloquinol		1 " 1,240	1 " 1,740
Thymoquinol		1 " 330	1 " 1,100
<i>The aldehydes</i>			
Formaldehyde	(i)	1 " 17,300	1 " 17,300
	(ii)	1 " 16,300	1 " 16,300
	(iii)	1 " 16,300	1 " 16,300
Glyoxal		1 " 466	1 " 900
<i>The amines</i>			
Aniline	(i)	1 " 355	1 " 250
	(ii)	1 " 333	1 " 225
Benzylamine		1 " 1,700	1 " 807
Phenylethylamine		1 " 1,340	1 " 1,340
Hydroxylamine hydrochloride	(i)	1 " 11,000	1 " 42,000
	(ii)	1 " 10,000	1 " 10,000
<i>The quinones</i>			
Benzoquinone		1 " 6,000	1 " 10,000
<i>Inorganic compounds</i>			
HgCl ₂	(i)	1 " 200,000	1 " 450,000
	(ii) less than	1 " 50,000	1 " 410,000
	(iii)	1 " 33,300	1 " 200,000
Cd SO ₄	less than	1 " 8,400	1 " 98,000
NaCl	(i)	1 " 15.4	1 " 15.4
	(ii)	1 " 19.0	1 " 15.0
(NH ₄) ₂ SO ₄	(i)	1 " 7.7	1 " 7.7
	(ii)	1 " 9.5	1 " 9.5
<i>Tellurium compounds</i> [Morgan, Cooper and Burt, 1924].			
Tellurium dipropionyl-methane		1 in 250,000	1 in 9,000,000

cyaneus. In the case of the aliphatic alcohols, this, however, does not hold, and the homologous series behaves quite consistently with both organisms. In ascending the series of phenols, however, whilst in the case of *B. coli*

germicidal action increases to a marked degree, with *B. pyocyaneus* there is only a very slight increase. The difference is so significant that thymol, which is eight times as active as phenol against *B. coli*, is only very slightly more efficacious than phenol with *B. pyocyaneus*. Exactly analogous results have been obtained with the alkyl derivatives of aniline.

In ascending the series of the homologous quinols, moreover, with *B. coli*, there is only a moderate increase in bactericidal power, while with *B. pyocyaneus*, there is actually a very marked diminution in efficacy. Consequently, thymoquinol, as in the case of thymol, is approximately four times as active against *B. coli* as against *B. pyocyaneus*.

There is no doubt that alkyl substitution in aromatic compounds has a very anomalous effect on germicidal power when the latter bacillus is used as the test organism. As a consequence, thymol and thymoquinol behave exceptionally in attacking *B. coli* more rapidly than *B. pyocyaneus*, and their specific action as physico-chemical germicides is thus obscured.

It is noteworthy that the three cresols have a higher germicidal power than the isomeric benzyl alcohol.

Sodium chloride, ammonium sulphate, and monochlorohydrin which may also be regarded as physico-chemical germicides, although they do not exhibit the preferential action of the above alcohols and phenols, yet differ from the majority of chemical germicides in exerting approximately an equal effect on the two organisms. In this respect they approach the exceptional case of formaldehyde referred to above.

It is interesting to observe that the amines examined, e.g. aniline and benzylamine, appear to belong to the class of physico-chemical germicides, as they exert a selective action upon *B. pyocyaneus*, in this respect resembling the analogous hydroxy compounds, phenol and benzyl alcohol.

Picric acid, however, is a more active germicide against *B. coli* than against *B. pyocyaneus*, and thus differs from the alcohol and phenol group of disinfectants.

Several investigators have drawn an analogy between the action of heat and alcohol upon proteins; Lepeschkin [1923] in fact has shown that the addition of alcohol increases the velocity of heat-denaturation of proteins. We therefore anticipated that *B. pyocyaneus*, being selectively attacked by alcohol and phenol, would be more susceptible to the action of hot water than *B. coli*. Filtered cultures (24 hours in broth) of *B. coli* and *B. pyocyaneus* were therefore heated in the same thermostat at 56° and sub-cultures made into fresh broth at regular intervals. It was found that, whilst a period of 75 minutes was required for the complete destruction of *B. coli*, *B. pyocyaneus* was killed off in the much shorter period of 15 minutes.

Quantitative experiments involving counting on agar plates were next carried out. Diluted filtered cultures of the two organisms were heated at 56°, each culture being provided with a stirrer in order to diminish the errors due to sampling, and sub-cultures were made at convenient intervals into

agar-plates. The latter were incubated at 37° for 48 hours, and the colonies then counted.

The results were as follows:

Table III.

Time at 56°, mins.	<i>B. coli</i> , Nos. per drop	Time, mins.	<i>B. pyocyaneus</i> , Nos. per drop
0	28,200	0	24,000
2	4,920	2	7,000
4	3,450	5	200
7	720	6	30
12	45	8	25
14	35	11	3
18	18		
22	2		
27	1		
32	1		

The above observations thus confirm the preliminary tests. It is evident that *B. pyocyaneus* is considerably more susceptible to the action of heat than *B. coli*, and the results as a whole strongly suggest a very close similarity in the mechanism of disinfection by heat and alcohol or phenol.

SUMMARY.

1. Several compounds, e.g. nitroso-compounds, quinone, aldehydes, salts of heavy metals, hydroxylamine hydrochloride, which react with the constituents of protoplasm and can therefore be classified as chemical germicides, exert a more powerful bactericidal action upon *B. coli* than on *B. pyocyaneus*. Formaldehyde behaves exceptionally in attacking both organisms with equal readiness.

2. The aliphatic alcohols, benzyl alcohol, phenol, chlorophenols, quinol, and toluquinol differ from the foregoing compounds in attacking *B. pyocyaneus* more readily than *B. coli*. In the case of quinol, the selective germicidal action is so marked that this substance is four times as active against *B. pyocyaneus* as against *B. coli*.

3. The above observations show that, whilst *B. pyocyaneus* is very resistant to the action of *chemical* disinfectants, it is selectively attacked by the germicides of the so-called physico-chemical type, e.g. alcohol and phenol, the bactericidal action of which is associated with some specific effect upon the colloidal condition of the bacterial protoplasm.

4. *B. pyocyaneus* is also considerably more susceptible to the action of heat than is *B. coli*. The latter observation supports the view that there is a fundamental similarity in the germicidal action of heat and of phenol or alcohol.

5. The results as a whole afford evidence of a very uncommon case of selective bactericidal action, which differentiates between the chemical and physico-chemical classes of germicides, and also suggest that the colloidal

structure of *B. pyocyaneus* is characteristically susceptible to the destructive effect of heat and denaturing substances.

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