Proceedings of the Iowa Academy of Science

Volume 26 | Annual Issue Article 10

1919

The Resistance of Streptococci to Germicidal Agents

Henry Albert
The State University

THE RESISTANCE OF STREPTOCOCCI TO GERMICIDAL AGENTS

HENRY ALBERT

During the past year and a half, streptococci have assumed an unusual role as causes of disease. They have been the cause of most of the serious infections of war wounds. They were almost entirely responsible for the severe epidemics of pneumonia and empyema which occurred in many places, especially military camps during the winter of 1917-18, and together with the pneumococcus were apparently the cause of most of the fatalities during the recent epidemic of influenza.

This unusual prominence of the streptococci was gained in part by wide distribution of virulent forms and probably also in part, by an increase in the virulence of the organism.

It is therefore a matter of great importance to determine if it is possible to destroy these chained cocci as they occur both in the normal body and in connection with the lesions of disease, without at the same time causing any great injury to the living tissue.

To date but few tests of this kind have been made. This is due to the fact that streptococci are difficult to cultivate and require special, not easily prepared media, for their recognition in plates. The more important contributions to this subject to date have been made by Lingelsheim[1] and by Post and Nicoll[2].

The purpose of the research here reported was to determine the germicidal effects of various chemical agents on streptococci with the hope of finding the most effective germicides in the presence of albuminous fluids such as are represented by the various fluids of the body.

TECHNIC

1. Exactly 5 c. c. of each of a number of dilutions of the germicide are measured into as many tubes. The same pipet may be used for the whole series by beginning with the lowest dilution. Tubes should be marked and placed in regular order in the racks.

2. With a sterile pipet, from 0.1 to 0.5 c. c. (to be varied as may be necessary) of a twenty-four-hour broth culture of the organism is added to each of the above test tubes at intervals of

ten minutes. Nutrient bouillon is used, being made with Liebig's beef extract and Witte's peptone in the usual manner and giving a reaction of exactly 1.0.

3. One-fourth minute after the "germicide" tube has had the culture added, a subculture is made from each tube in the series of dilutions by transplanting one loopful to a tube of 10 c. c. sterile broth. (The loops used were of No. 23 U. S. standard gage platinum wire, each loop being 4 mm. in diameter.)

4. Immediately after the broth tube, inoculated with a loopful of the culture treated with the germicide, has been made, 1 c. c. of this broth culture is plated with 10 c. c. of blood agar. It is mixed in a test tube before being poured into the Petri dish.

5. At 1-2, 1, 2, 5, 10, 30 and 60 minute intervals cultures are made in the same way as in Direction 3.

6. The cultures should be incubated at 37°C. for twenty-four to seventy-two hours. "Record" growths usually develop in forty-eight hours.

CONTROLS

1. From 0.1 c. c. to 0.5 c. c. (as used in the foregoing) of the same twenty-four-hour broth culture of the micro-organism used in the experiment should be placed in exactly 5 c. c. of plain broth.

2. One "standard" loopful of the foregoing diluted culture should be transferred to a 10 c. c. broth tube. This will be the "broth control."

3. One c. c. of the "broth control" should be transferred to a 10 c. c. tube of blood agar. The agar tube should be poured into a Petri dish. This will be the "agar control."

4. The cultures should be incubated at 37°C. for twenty-four to seventy-two hours. Record growths after the same period of incubation as used for the experiment.

As a rule, aqueous solutions of the germicide were used. To test the effect of the germicide on the micro-organism in the presence of albuminous material, we also used blood, serum water and dilute serum water. The blood used was defibrinated sheep blood. The serum water was prepared by mixing one part of beef blood serum with three parts of distilled water and sterilizing the mixture on three successive days in the Arnold steam sterilizer. Dilute serum water was prepared in the same way, except that one part of blood serum was used to ten parts of water.

Blood agar was prepared by the addition of 10 per cent of defibrinated sheep blood to plain agar and by the process of ad-

justing the temperature to 0.5 reaction, with phenolphthalein as the indicator. From 10 to 15 c. c. of the blood agar were poured into each Petri dish.

FINDINGS

The results of our experiments have been placed in Tables 1 and 2. Table 1 shows the effects of various germicides in reducing the number of streptococci or entirely destroying them after periods of exposure varying from one-fourth minute to one hour. Table 2 indicates the shortest time in which all streptococci of a given culture were killed by various germicides as well as a list of those germicides which failed to kill all the streptococci at the end of one hour.

The letters "h" and "v" in the column under "Types of Streptococci" refer to "hemolyticus" and "viridans" respectively. The plus and minus signs in the column under "G" refer to the presence or absence of growth in the broth tubes. The figures in the column under "No." refer to the number of bacteria living in a given volume (as explained in the discussion of the technic), after the disinfectant had been permitted to act for a given length of time, as represented in Table 1. The number of bacteria was determined by the number of colonies that developed on the blood-agar plates. In order that the figures may be made comparable, they have been reduced to a basis of a control of 1,000 colonies.

TABLE NO. 1 SHOWS THE EFFECTS OF VARIOUS GERMICIDES IN REDUCING THE NUMBER OF STREPTOCOCCI OR ENTIRELY DESTROYING THEM AFTER PERIODS OF EXPOSURE VARYING FROM ONE-FOURTH MINUTE TO ONE HOUR.

Reagent	Dilution	Type of Streptococcus	¼ G	¼ No.	½ G	½ No.	1 G	1 No.	2 G	2 No.	5 G	5 No.	10 G	10 No.	30 G	30 No.	60 G	60 No.
Alcohol	50%	v	+	5	+	0	+	0	+	2	+	2	—	0	—	0		
Alcohol	25%	v	+	260	....	.....	....	.....	....	.....	....	.....	....	.....	+	45	—	7
Alcohol	50%	h	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0
Alcohol	75%	v	+	2	+	10	+	2	—	0	—	0	—	0				
Alcohol	95%	h	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0
Boric acid	4%	v	+	840	....	.....	....	.....	....	.....	....	.....	....	.....	+	400	+	115
Phenol	5%	v	....	.....	....	.....	+	80	+	87	+	87	+	15	—	0	—	0
Chloramin-T	0.2%	h	+	400	+	200	—	0	—	0	—	0	—	0	—	0	—	0
Chloramin-T	0.1%	h	+	700	+	500	+	300	—	0	—	0	—	0	—	0	—	0
Chloramin-T	0.05%	h	+	900	....	.....	+	700	....	.....	+	200	+	100	—	0	—	0
Chloramin-T	0.2% in serum water	h	+	1,000	+	650	+	350	+	95	—	0	—	0	—	0	—	0
Chloramin-T	0.2% in blood	v	+	950	+	950	+	600	+	900	+	900	+	950	+	900	+	950
Chloramin-T	1% in serum water	h	+	380	+	40	—	0	—	0	....	0	....	0	....	0		
Chloramin-T	0.5% in serum water	h	+	390	+	200	+	300	....	.....	+	130						
Dakin's solution	100%	v	—	3	....	.....	....	.....	....	.....	....	.....	....	.....	—	6	—	6
Dichloramin-T	0.5%, 1%, 2%	h	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0
Ethylhydrocuprein hydrochlorid	1:500	v	....	.....	....	.....	+	200	....	.....	....	.....	....	.....	+	200	+	500
Glycerin	10%	v	+	89	....	.....	....	.....	....	.....	....	.....	....	.....	+	62	+	89
Hydrogen peroxid solution	1%	v	....	.....	....	.....	+	810	+	370	+	860	+	430	—	20		

Iodine	1:2,000	h	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0
Iodine	1:1,000 in diluted serum water	h	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0
Iodine	1:10,000 in diluted serum water	h	+	721	+	735	+	714	+	1,078	+	1,330	....	.....	+	749	+	1,050
Menthol	1:1,000	v	....	.....	....	.....	+	880	....	.....	....	.....	....	.....	+	600	+	140
Mercuric chlorid	1:1,500	v	....	.....	....	.....	+	620	+	150	....	.....	—	0	—	0	—	0
Mercuric chlorid	1:65,000	v	....	.....	....	.....	+	910	+	840	+	840	—	385	—	7	—	7
Quinin sulphate	1:350	v	....	.....	....	.....	+	405	....	.....	....	.....	....	.....	—	18	—	27
Sodium benzoate	....	.....	....	.....	....	.....	+	840	....	.....	....	.....	....	.....	+	800	+	392
Sulphuric acid	1:150	v	....	.....	....	.....	—	37	—	37	—	0	—	40	—	12	—	12
Thymol	1:750	h	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0
Thymol	1:1,000	h	+	25	+	8	—	0	—	0	—	0	—	0	—	0	—	0
Thymol	1:1,000	v	+	540	+	290	+	110	+	161	+	23	+	2	+	2	+	0
Thymol	1:2,000	h	+	225	+	100	—	0	—	25	—	0	—	0	—	0	—	0
Thymol	1:750 in serum water	h	+	100	....	.....	—	14	—	6	—	0	—	0	—	0	—	0
Thymol	1:750 in blood	h	+	210	+	260	+	250	....	.....	+	260	+	210	+	200		

h=Streptococcus hemolyticus
v=Streptococcus viridans
+=Growth (bacteria not all killed).
—=No growth (bacteria all killed).
Figures represent number of bacteria of a total of 1,000 not killed as indicated by number of colonies which developed.

TABLE NO. 2 INDICATES THE SHORTEST TIME IN WHICH ALL STREPTOCOCCI OF A GIVEN CULTURE WERE KILLED BY VARIOUS GERMICIDES AS WELL AS A LIST OF THOSE GERMICIDES WHICH FAILED TO KILL ALL THE STREPTOCOCCI AT THE END OF ONE HOUR.

Reagents	Dilution (in water) unless otherwise specified	Type of streptococcus	Shortest time tried in min. in which all streptococci were killed	Streptococci not all killed in 1 hour
Alcohol	50%	h	one-fourth min.	+
Alcohol	50%	v	ten min.	
Boric acid	1–25	v		+ numerous
Carbolic acid	1–200	v	thirty min.	
Chloramin–T	0.2%	h	one min.	
Chloramin–T	0.1%	h	two min.	
Chloramin–T	0.2% in serum water	h	five min.	
Chloramin–T	0.2% in blood	h		+ numerous
Dakin's solution	100% and all sol. below	v		+ few
Dichloramin–T	0.5%	v and h	one-fourth min.	
Ethylhydrocuprein hydrochlorid	1–500	v		+numerous
Iodine (Tr.)	1–2000 in water	h	one-fourth min.	
Iodine (Tr.)	1–2000 in dilute serum water	h	one-fourth min.	
Mercuric bichloride	1–1500	v	ten min.	
Quinine sulphate	1–350	v		+ few
Sulphuric acid	1–150			+ few
Thymol	1–750	h	one-fourth min.	
Thymol	1–750 in serum water	h	five min.	
Thymol	1–750 in blood	h		+ numerous

SUMMARY AND CONCLUSIONS

1. Streptococci are probably the most important disease-producing bacteria.
2. The frequency with which streptococci are found in the mouth, throat and in connection with wounds, enables them to be acted on quite directly by germicidal agents.
3. As yet very little work has been done in testing the effect of germicides on streptococci.
4. The most efficacious of the germicides tested are as follows: alcohol, 50 or more per cent; chloramin-T; dichloramin-T; iodin; mercuric chlorid and thymol.
5. Certain commonly employed germicides as boric acid and

hydrogen peroxid solution were found to be of very little value.

6. Iodine is very effective even in a high dilution and in the presence of a moderate amount of albuminous material. In a solution of 1:2,000, it destroyed all streptococci suspended in water and in a solution of 1:1,000, all of them suspended in an albuminous solution within one-fourth minute. It would seem that it would be advisable to use very much more dilute solutions of iodine than are ordinarily employed, especially since the more concentrated solutions are both irritating and destructive of tissue cells.
7. The presence of albuminous fluid very markedly reduces the germicidal effect of a number of agents, of which chloramin-T and thymol may be mentioned as examples.
8. There does not seem to be a "specific" germicide for streptococci.

Acknowledgement is here made to the effect that most of the technical work of this piece of research was done by Miss Margaret Taylor.

It is planned to continue this investigation with the hope of finding one or more germicides which will destroy streptococci without having any or at least only relatively slight harmful effects on the tissue cells.

BACTERIOLOGICAL LABORATORY,
THE STATE UNIVERSITY.

REFERENCES

1. **Lingelsheim, W. V.:** Streptokokken, Handbuch der Pathogenen Mikroorganismen, von Kolle and Wassermann, 4, 465, also,
Lingelsheim, W. V.: Aetiologie und Therapie der Streptococcus Infektionen, Beitr. z. exper. Therap., 1899, p. 1.
2. **Post, N. E. and Nicoll, H. K.:** The Comparative Efficiency of Some Common Germicides, The Journal A. M. A., Nov. 5, 1910, p. 1635.