EXPERIMENTS ON DISINFECTION OF *BACILLUS PESTIS* WITH "CYLLIN," "ABSOLUTE PHENOL," AND "FORMALIN."

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THE following experiments were undertaken to test and compare the relative actions of "cyllin," "phenol," and "formalin" on *Bacillus pestis* of a virulent strain. This had been originally derived from the bubo of a fatal case of bubonic plague in a man in Cardiff in 1901. The strain had been kept up in the laboratory by continued subcultures till the commencement of this year, when it was used for infecting rats. A trace of an agar culture of recent date (48 hours at 37° C.) inoculated into a superficial incision of the skin of a rat, caused typical fatal plague within three days. The culture actually used in the experiments to be described here was derived from the spleen of a rat dead on March 23rd, 1904, which rat had been cutaneously inoculated on March 20th, 1904. In all our tests of a 48 hours' old agar culture—copious growth over the whole of the sloped surface (6 centimetres by 2 centimetres)—an emulsion was made with sterile distilled water. The emulsion was a strongly turbid fluid. A single platinum loop of this emulsion spread over a sloped agar surface brought forth innumerable colonies of the *B. pestis*. In all instances to 5 cc. of the disinfectant contained in a sterile test tube, five drops (from a definite drop bottle) of the plague emulsion were added and well shaken, and after the required time of exposure cultures were made; in all instances three loops of the medicated fluid being spread out over a sloped surface of gelatine or agar, or added to nutrient broth, as the case might be. The culture tubes were then incubated at 21° C. (gelatin), or at 37° C. (agar or broth), and inspected from time to time until for some days no further change was noticeable. Amongst the several dozen cultures thus made, in no single instance was there any accidental or stray microbe observed; the tubes contained either no growth at all, or they contained colonies of the *B. pestis* in pure culture only, in varying numbers, as will presently be described. In this connection it will be necessary to bear in mind that three platinum loops of the mixture (5 cc. of the disinfectant and 5 drops of the emulsion of *B. pestis*), yielded in the positive instances innumerable colonies of *B. pestis* in the culture tubes. This disposes at once of an objection that might possibly be raised, viz., that the amount of disinfectant carried over from the mixture to the culture tubes might inhibit the subsequent growth of the microbe, although the latter might still be alive. It also disposes of a further

criticism, viz., that the number of microbes carried over by three platinum loops might not be large enough. As just mentioned, in the fully positive instances the whole culture surface was covered with crowds of colonies.

Series I.—March 25th, 1904.

A.—Watery solution of pure phenol	..	..	1 in 200
B.—Watery solution of pure phenol	..	..	1 in 100
C.—Watery solution of formalin (40 per cent)	..	..	1 in 100
D.—Watery solution of formalin (40 per cent)	..	..	1 in 50

Time of exposure of *B. pestis*, 5, 10, and 15 minutes.

Cultures after exposure were made on sloped gelatin (21° C.), and inspected on April 5th, with the following result:—

1.—Phenol 1 in 200, 5 minutes exposure.	Innumerable colonies—
confluent mass
10 " "	" "
15 " "	" "
2.—Phenol 1 in 100, 5 minutes exposure.	Innumerable colonies
10 " "	About 7 doz. colonies
15 " "	6 colonies
3.—Formalin 1 in 100, 5 minutes exposure.	Innumerable colonies
10 " "	" "
15 " "	" "
4.—Formalin 1 in 50, 5 minutes exposure.	Innumerable colonies
10 " "	" "
15 " "	" "

Series II.—March 28th, 1904.

<i>A.</i> —Watery distribution of cyllin	..	..	1 in 1,500
<i>B.</i> — " " " "	..	..	1 in 1,000
<i>C.</i> — " " " "	..	..	1 in 500

Time of exposure, 5, 10, and 15 minutes.

Cultures after exposure were made on sloped gelatin (21° C.) and inspected on April 10th, when all tubes were found to be free of any growth.

Series III.—April 8th, 1904.

<i>A.</i> —Watery distribution of cyllin	..	..	1 in 1,500
<i>B.</i> — " " " "	..	..	1 in 1,000

Time of exposure, 5, 10, and 15 minutes.

Cultures after exposure were made on sloped agar and in broth, and incubated at 37° C., inspection being made on April 16th, 1904.

Result: All tubes free of any growth.

Series IV.—April 7th, 1904.

<i>A.</i> —Watery solution of phenol	..	..	..	1 in 80
<i>B.</i> — " formalin	..	..	..	1 in 30

<i>C.</i> —Distribution of cyllin	..	..	..	1 in 2,000
<i>D.</i> —	„	„	„	1 in 1,800
<i>E.</i> —	„	„	„	1 in 1,600

Time of exposure, 5, 10, and 15 minutes.

Cultures were made on gelatin (21° C.) and agar (37° C.) and inspected on April 16th, with the following result:—

1.—Phenol 1 in 80, 5 minutes exposure. On gelatin 3 doz. cols.

On agar „ „

10 „ „ In both tubes no growth

15 „ „ „ „ „

2.—Formalin 1 in 30. 5 minutes exposure. Crowds of colonies in both tubes

10 „ „ Numerous colonies

15 „ „ About 5 doz. colonies

3.—Cyllin 1 in 2,000, 5, 10, and 15 minutes exposure. All tubes free of growth

1 in 1,800 „ „ „ „ „

1 in 1,600 „ „ „ „ „

Series V.—April 11th, 1904.

A.—Watery distribution of cyllin 1 in 2,400

B.— „ „ „ 1 in 2,200

Time of exposure, 5, 10, and 15 minutes.

Cultures were made on agar (37° C.) and gelatin (21° C.), and inspected on April 18th with this result:—

1.—Cyllin 1 in 2,400, 5 minutes exposure. Gelatin tube had one

colony of *B. pestis*.

The agar tube had a

small amount of

growth in the con-

densation water; this

was allowed to spread

over the sloped surface

of the agar, with the

result that copious

growth of *B. pestis*

covered the surface

next day.

10 „ „ No growth in any tube

15 „ „ „ „ „

2.—Cyllin 1 in 2,200, 5 minutes exposure. All tubes free of any growth

10 „ „ „ „ „

15 „ „ „ „ „

From these experiments it follows : (1) That formalin even 1 in 30 failed to disinfect *B. pestis* in 15 minutes ; (2) That phenol 1 in 80 failed to disinfect *B. pestis* in 5 minutes, but did disinfect in 10 minutes ; and (3) that cyllin 1 in 2,400 failed in 5 minutes, but succeeded in 10 minutes.

From the following tables it will be seen that :—

(1). Phenol 1 in 80 is a stronger disinfectant for *B. pestis* than formalin 1 in 30, and

(2). Phenol 1 in 80 stands in about the same position as cyllin 1 in 2,400, inasmuch as both failed to completely devitalize the *B. pestis* in 5 minutes, though they both succeeded in doing so in 10 minutes. Expressed in a different form, cyllin possesses for watery emulsion of *B. pestis* a disinfecting power which is more than 27·5 (in fact about 30) times as great as that of absolute phenol.

TEST No. 1. (April 22nd, 1904).

B. PESTIS, 48 HOURS AGAR CULTURE AT 37° C.

ROOM TEMPERATURE 15°-18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant.			Sub-Culture.		Remarks.
		5	10	15	Period of Incubation.	Temperature.	
Formalin (40%)	1: 100	+	+	+	7 days	37° C.	Copious growth
	1: 50	+	+	+	"	"	"
	1: 30	+	+	+	"	"	Slight reduction in 15 minutes
Pure Phenol ...	1: 80	+	-	-	"	"	Reduction in 5 minutes

∴ Carbolic Acid Co-efficient of Formalin is under 0·37.*

TEST No. 2. (April 22nd, 1904.)

B. PESTIS, 48 HOURS AGAR CULTURE AT 37° C.

ROOM TEMPERATURE 15°-18° C.

Sample.	Dilution.	Time culture exposed to action of disinfectant.			Sub-Culture.		Remarks
		5	10	15	Period of Incubation.	Temperature.	
Cyllin ...	1: 2400	+	-	-	7 days	37 C.	Great reduction in 5 minutes
	1: 2200	-	-	-	"	"	-
	1: 2000	-	-	-	"	"	-
	1: 1800	-	-	-	"	"	-
Pure Phenol ...	1: 80	+	-	-	"	"	Reduction in 5 minutes

∴ Carbolic Acid Co-efficient of Cyllin is 30·0.

* A similar co-efficient for Formalin was obtained with *B. typhosus*, by Mr. Ainslie Walker. Jan., 1904.—ED.