8544 C

Bactericidal Power of Dried Painted Surfaces.* (I).

EUGENIA VALENTINE. (Introduced by K. G. Falk.)
From the Department of Preventive Medicine, New York University College of Medicine.

The development of a paint which has bactericidal properties after drying has been reported recently by Wetchler, Lewis and Battline.[1] This paint has, as the binder, a 4% halogenated oil base. The present study deals with bactericidal tests on this type of paint after prolonged periods of drying in comparison with a number of ordinary commercial paints bought in the open market. The cultures used for testing have been *B. typhosus*, "Hopkins" (U.S. Food and Drug Administration strain), and *Staphylococcus aureus*, 4 strains, namely, No. 209 (U.S.F.D.A. strain), No. 3 (stock strain of the Bureau of Laboratories, N.Y.C. Dept of Health), and 2 freshly isolated from boils. Work on bactericidal paints has been reported by Portier and Kling,[2] Trouissant,[3] and Vallée.[4]

Two methods were used for determining bactericidal action as judged by the lack of growth on media. The method on which the majority of results given in this paper were based, consisted in the use of painted glass rods, 10 mm. in diameter, which were allowed to dry in the air for various periods of time. Baking at 100° C. and 125° C. was employed to simulate longer periods of air-drying; this method of aging is a common practice in paint technology. For the tests, the rods were immersed in test tubes containing 4 cc. of saline (0.85%) suspension of the culture (18 hour agar slant) and kept at room temperature for a given time. The rods were then removed and 0.2 cc. of the culture suspension placed on the surface of extract agar plates. Observation of the amount of growth was made after 18 and 42 hours' incubation at 37° C. Prolonged incubation for 4 days did not show any appreciable increase in the number of colonies. Dilution pour plates were

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1 Wetchler, S., Lewis, A., and Battline, F., *Paint and Varnish Production Manager*, 1936, **14**, 12.

2 Portier, P., and Kling, A., *Bull. Acad. de Med.*, 1931, **406**, 305.

3 Trouissant, *Bull. Acad. de Med.*, 1933, **109**, 448.

4 Vallée, *Chemie et Industrie*, 1934, Special No. 962-968.

made in a number of instances to eliminate the possibility of an inhibitory factor which would be removed by further dilution. The density of the bacterial suspension used in each test was determined by comparison with barium sulfate standards and by plate count made from serial dilution in salt solution.

In the second method 0.1 cc. of the saline culture suspension was placed on a square inch area of a painted tin disc, and separated into small drops with the aid of a loop. The discs were then placed in petri dishes containing a layer of filter paper moistened with 1 cc. of water and allowed to stand at room temperature for 6 hours; then 0.3 cc. of sterile salt solution was pipetted onto the area, rinsed up and down 3 times, and placed on the surface of extract agar plates. These plates were then incubated and observed for growth as in method 1.

Experiments with *B. typhosus.* Method 1. The results of the tests of 2 halogenated paints, A and C, and the 10 commercial paints, D through P, with *B. typhosus* (*B.t.*), carried out according to method 1, are summarized in the table. The 3 commercial paints, D, E, F, were used for comparison in the majority of tests; later, 7 additional paints were studied. Painted rods were tested after air-drying for periods from 2 days to 9 weeks, and after baking at 100° C. and 125° C. for periods up to 6 hours. The results of these experiments showed that the dried painted films of paints A and C exerted a marked bactericidal effect upon saline suspensions of *B.t.* in the test tube, as judged by the lack of growth on agar plates. The painted surfaces were still effective after air-drying for 9 weeks and after baking for 6 hours at 125° C., an approximate equivalent of 6 years of air-drying.† In all of the tests with paints A and C, the growth was either completely inhibited or the initial viable population of the culture was reduced from 15-80 million colonies per cc. to 250-1000 per cc.

Of the 10 commercial paints, 7 had bactericidal power 2 days after painting but by the tenth day only 4 were slightly germicidal. When tested 6-7 weeks after painting, paints D and O remained somewhat effective; and, after baking at 100°C. for 6 hours, O alone showed very slight bactericidal action.

Method 2. A limited number of experiments were performed with painted tin discs after air-drying for 2 days and 7-8 weeks, and baking at 100°C. for 6 hours, an approximate equivalent of

†The rate of a chemical reaction is increased from 2 to 3 times for every 10 degree rise in temperature Centigrade. The average is used here to arrive at the approximate time-equivalent of baking and air-drying.

380 days of air-drying. The results were as follows: after 2 days of air-drying, paints A,C,D,F,G,H,K,L, and O were entirely bactericidal; paints E,J, and P showed no growth inhibition. When air-dried for 7-8 weeks, A,C,D,G, and O were strongly bactericidal while the rest showed no, or very slight, inhibition of growth. After 6 hours at 100°C. the halogenated paints A and C were completely bactericidal and all of the commercial paints were devoid of germicidal activity. These results with the painted discs, air-dried for 2 days, 7 weeks or baked at 100°C. for 6 hours coincided closely with those obtained with the rods, the only variation being the evidence of germicidal power by paints D and O when air-dried for 7 weeks.

Experiments with *Staphylococcus aureus.* Method 1. The greater resistance of *Staphylococcus aureus* (*S. a.*) to disinfectants, as shown in phenol coefficient tests, was also apparent in the preliminary tests of this organism with the halogenated paints. These tests showed that the time of exposure (6 hours) effective in the case of *B.t.* was insufficient for *S.a.*

Twenty-two experiments were carried out with *S.a.* No. 209 and No. 3 in which the time of exposure of the painted rod and culture suspension varied from 16-24 hours and the strength of the culture suspension from 15-115 million colonies per cc. The rods were tested after air-drying for periods up to 7 weeks and after baking at 100°C. for 45 minutes, 2 and 4 hours. The results of these tests did not show the consistent and marked bactericidal effect that had been found with *B.t.* The halogenated paints A and C were strongly germicidal in 16 and 15 experiments, respectively, out of 22. In all of these tests, paints A and C showed greater action upon the bacteria than the commercial paints E and F, but were only slightly better than paint D. Preliminary work on a slight modification of paints A and C has shown encouraging results with *S.a.* No. 209 after 6 hours' exposure. Further experiments on this paint are in progress.

Wetchler, Lewis, and Battline[1] reported strong and consistent bactericidal action upon a staphylococcus isolated from the throat. This strain was found to be somewhat atypical in growth characteristics and therefore it was not included in the experiments presented here.

Summary and Conclusions. Two halogenated paints and 10 commercial paints were tested for bactericidal action after drying in the air for periods up to 9 weeks and after baking at 100°C. and 125°C. for periods up to 6 hours, approximate equivalents of 380

TABLE I.

Bactericidal Tests of Painted Rods—*B. typhosus*.

Method of Aging		Air-Dried				100° C. Baked			125° C Baked		
Time of drying or baking		2 days	7-10 days	6-7 wks.	9 wks.	6 min.†	1,4-hr.	6 hr.	30 min.	1 hr.	6 hr.
Strength of bacteria in millions, per cc.*		50-140	80-150	80	68	115	80	80	25	25	15
Paint	Description										
A	Halogenated	0	0	0-tr	tr	0	0	0	tr	0	tr
C	”	0	0	0-tr	0	0	0	0-tr	tr-+	0	0-tr
D	Gloss ivory	0-+1	++	+1	+1	+1	++	++	++	++	++
E	Enamel ivory	++	++	++	++	+1	++	++	++	++	++
F	Semi-gloss ivory	0-+1	+1-++	++	++	+1	++	++	+1	++	++
G	Gloss white	0	++	++	++			++			
H	China gloss white	tr	+1-++	++				++			
J	Gloss white	++	++	++				++			
K	Inside gloss white	tr	++	++				++			
L	Gloss white	0	++	++				++			
O	Inside gloss white	0	tr	tr-+1				+1			
P	Interior gloss white	++	++	++				++			
Control	No paint	++	++	++	++	++	++	++	++	++	++

*Colony count of culture suspension in which rods were immersed.

†Approximate equivalent time of air-drying:

At 100° C,

6 min. = 6⅓ days
15 ” = 15½ ”
45 ” = 47 ”
4 hr. = 253 ”
6 ” = 380 ”

At 125° C,

30 min. = 6⅔ mo.
1 hr. = 400 days
4 ” = 4½ yr.
6 ” = 6½ ”

Explanation of Symbols

0 = No growth on surface agar plates after 18 hr. incubation; none or less than 50 colonies after 42 hr. incubation.

tr (trace) = No growth or very slight growth after 18 hr. incubation; between 50 and 200 colonies after 48 hr. incubation.

+ = Slight growth after 18 hr. incubation; between 200 and 800 colonies after 42 hr.

+1 = Moderate growth after 18 hr. incubation.

++ = Heavy growth after 18 hr. incubation, equal to control.

The large majority of the tests tabulated were carried out on duplicate rods. Where 2 readings are given, the results of the 2 rods were different.

days and 6 years of air-drying respectively. The results of the experiments showed that glass rods, painted with the halogenated paints, after air-drying or baking for the time and temperature indicated, exerted a strong bactericidal effect upon saline suspensions of *B. typhosus,* when exposed for 6 hours at 20°C., as judged by the amount of growth on agar plates. Of the 10 commercial paints tested in the same manner, 7 were bactericidal 2 days after painting, but when tested 8 days later 9 had almost or completely lost bactericidal power; at the end of 6 weeks no appreciable germicidal property was manifested in any of them. Painted tin discs, air-dried for 2 days or 7 weeks, or baked at 100°C. for 6 hours gave results which coincided closely with those obtained with the rods.

Experiments with *Staphylococcus aureus* exposed to painted rods for 16-24 hours showed that the painted surfaces of the halogenated paints air-dried for periods up to 7 weeks, or baked at 100°C. for 45 minutes, 2 and 4 hours, were moderately but not consistently bactericidal. They were superior to the 3 commercial paints tested in comparison.

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Permeability of Germinal Vesicle of the Starfish Egg to Water.

LYLE V. BECK AND HERBERT SHAPIRO.
(Introduced by Robert Chambers.)
Marine Biological Laboratory, Woods Hole, Mass., and Physiological Laboratory, Princeton University.

While an enormous amount of research has been carried out on the permeability of the external cell membrane, very little consideration has been given to the permeability of the nuclear membrane. In fact, only one paper, that of Monne[1] on diffusion of microinjected dyes from cytoplasm to nucleus, has come to our attention.

A few preliminary experiments, carried out at the end of the past season at Woods Hole, indicate the possibility of making quantitative and extended studies on the permeability to water of the nuclear membrane in permanently immature eggs of the starfish, *Asterias forbesii.* The great advantage of this cell over others

1 Monne, L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1197.