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INVESTIGATION ON AREA DISINFECTION WITH BETA-
PROPRIOLACTONE IN AEROSOL FORM

(These investigations were conducted with the support of the
German Research Association.)

From the Hygiene Institute of Mainz University (Prof. Dr.
H. Kliewe, Director).

The effectiveness and applicability of disinfection agents in aero-
sol form are limited not only by the multiplicity of ways in which patho-
genic germs can be transmitted but also by the physical-chemical behavior
of the disinfecting vapors and aerosols (1, 7, 56). Disinfectants used in
the form of aerosols are therefore generally used exclusively for area air
disinfection which, however constitutes a valuable measure supplementing
exposure prophylaxis (1, 2, 3, 7, 26, 27, 34-40, 54, 57, 58). Aerosols
used nowadays can give us a more or less adequate disinfection of area
(room) air but unfortunately it cannot achieve an adequate infection
effect along surfaces /three-dimensional as against two-dimensional dis-
infection effect/. Only formalin aerosol does not follow this rule because
both the air and objects and surfaces can be disinfected as a result of
the formaldehyde gas which is released when formalin is atomized (8, 59).
This property is responsible for the extensive use fo formalin in the past
and in the present, that is, for purposes of room spraying as part of a
thorough final disinfection of sick rooms in hospitals and elsewhere. Of
all of the other substances tested for their suitability in room or area
air disinfection (triethylene glycol, triethylene glycol-containing pre-
parations, propylene glycol, preparations based on hexylresorcin, etc.)
none offered the advantages inherent in formalin, that is, good solubility
in water, easy volatility, relative harmlessness, and a good germ-killing
effect both in the air and along surfaces. Ethylene oxide gas, which has
come into use recently and which is employed primarily in other fields
(41, 42) is likewise unsuitable for room or area spraying for a number of
reasons (50). Final decontamination using formalin vapor or aerosol is
today prevented by a number of weighty reasons. The chief disadvantages
of the formalin method reside in the transportation of the equipment, in
the long period of time, that is, several hours, required to have the
agents take effect, and in the subsequent neutralization with ammonia
which again takes time. Final disinfection with formalin vapor is considered
a necessary evil particularly because of the overcrowding of hospitals and
the shortage of personnel. We therefore do not need to explain any further
why it is of the utmost importance for us to discover more convenient methods

for reliable room disinfection.

The discovery of the germ-killing effect of beta-propiolactone (BPL) during the vapor phase by Hoffman and Warshowsky (31) might therefore possibly constitute a valuable contribution to disinfection and sterilization in general and to room disinfection in particular.

Beta-propiolactone ($C_3H_4O_2$) has the structural formula CH_2—CH_2

$$O \text{———} C = O;$$

in concentrated form it is a colorless stable liquid with the following physical properties:

Specific weight	1.149
Boiling point	155° C
Melting point	-33.4° C
Vapor pressure (25° C)	3.4 mm Hg
Water solubility (25° C)	37 Vol. %

In watery solutions BPL is unstable and very quickly is hydrolyzed into nontoxic decomposition products; here the hydrolysis, measured in half-life periods, is a function of the temperature. In concentrated form, BPL remains unchanged for several years if kept at a temperature of 4° C; at higher temperatures (for instance, 5° C) it is polymerized within a few weeks. It reacts quickly with hydroxyl-, amino-, carboxyl-, sulfhydryl-groups and phenols. Inorganic salts, acids, and bases catalyze the polymerization of the liquid BPL or form new reaction products with it. Details on the chemical properties of BPL have been published by Gresham and associates, as well as by Bartlett and associates (9, 10, 15-25). The virus-killing, bacteria-killing, spore-killing, and fungicidal properties of BPL in watery solutions (4, 14, 28, 29, 30, 32, 43-48, 51, 52, 53, 55) make possible the successful disinfection of plasma, transplant tissues, vaccines and other biological material (Hartman, Kelly, Lo Grippo and associates). A report on the antimicrobial effectiveness of a german BPL preparation in vitro was made elsewhere (Husmann).

So far, BPL has been used for disinfection only in watery solutions; Hoffman and Warshowsky, however, were able to show -- in experiments on germ carriers to which adhered pathogenic staphylococci, respectively, spores of Bacillus subtilis -- that the substance is more effective than formaldehyde after aerolization and that it takes effect much more quickly and has less harmful accompanying effects. Employing similar experimental procedures, Dawson and associates (11, 12) achieved corresponding results in VEE-, small-pox, yellow fever, and psittacosis viruses and Rickettsia burneti.

In view of the importance of the prevention of the spread of micro-organisms through the air ("airborne infection") I conducted the first investigation of the antimicrobial property of BPL in aerosol form on microorganisms in room air and I also tested its disinfectant effects along surfaces.

Method

A. The investigation on the disinfectant effect of BPL in room air was conducted on the basis of a method suggested by Albrecht (5) and reported on elsewhere so that we need not go into a detailed description here. I will discuss only those points where I deviated from this method. The disinfectant effect of BPL aerosol was tested against artificial germ aerosols consisting of staphylococci (micrococcus pyogenes var. albus) and Coliphagen-(T_3). The germ suspension (18 hours with physiological NaCl solution in a ratio of 1:10 diluted bouillon culture, respectively, its filtrate) and the disinfectant (30% aqueous solution) (the beta-propiolactone was obtained from the Dr. Th. Schuchardt Company in Munich) were atomized by means of the centrifugal atomizer (8) and the quantity of the germs in the air was determined with the help of the so-called modified Moulton unit (6). In addition I determined the number of germs in the air also according to the sedimentation method using settling plates. During the experiment the temperature changed very little and the relative humidity in the test room, which had a volume of 35 m³, was kept relatively constant by means of a Defensor air humidifier with hydrostat; the fluctuations were very small. The experiment took place in the following manner:

Minutes After Start of Experiment	Measures Taken
0 - 3	Atomization of germ suspension
9 - 12	Settling plate A
9 - 14	Moulton Unit A
after 15	Atomization of water (empty experiment) respectively BPL solution
29 - 32	Settling plate B
29 - 34	Moulton unit B
44 - 47	Settling place C
44 - 49	Moulton unit C
59 - 62	Settling plate D
59 - 64	Moulton unit D
74 - 77	Settling plate E
74 - 79	Moulton unit D

A quantity of 0.5 ml of the washing liquid (sterile tap water) was -- concentrated and diluted in a ratio of 1:10 and 1:100 -- inoculated in a double deposit on nutrient agar plates and the germs (viruses) contained in 1 liter of air was calculated on the basis of the colonies which grew after 24-hour incubation. Of the phage-containing washing liquid I also spread 0.1 ml together with 0.1 ml E.-coli-bouillon in a similar manner on agar plates on which the phage holes could be counted on the next day. To prevent any possible bactericidal aftereffect of BPL in the washing water for the germ determination equipment I added 0.5% sodium thiosulfate in a parallel experiment in order to deactivate the agent. The evaluation of the experiment however did not reveal any difference with respect to the deposits not involving NA-thiosulfate; this appears to have been caused by the extraordi-

narily rapid hydrolysis of BPL in aqueous solutions. This is why I did not
have to conduct any further experiments involving the addition of NA-thio-
sulfate.

   B. To determine the disinfecting effect of BPL aerosol on surfaces,
I applied, to the top of a 6 X 6 cm large unwaxed sterile linoleum plate,
using sterile swabs, suspensions of 18-hour bouillon cultures of micrococcus
pyogenes var. albus, Micrococcus pyogenes var. aureus (SG 511), Escherichia
coli, as well as a culture filtrate with coliphagen-T_3 and I exposed the in-
fected substances for 15, 30, 45, 60, and 120 minutes to the action of a BPL
aerosol (0.5 mg pure substance/1 air). Untreated controls remained in sterile
Petri dishes for the corresponding periods of time until further use was made
of them. To control the disinfectant effect, I made moist swab skimmings of
the bacteria-infected treated and untreated surfaces, after the periods of
time mentioned earlier; I transferred these skimmings to agar plates and tu-
bules containing nutrient bouillon; these skimmings were then checked for
germ growth after 24-hour incubation at a temperature of 37° C. The swab
skimmings of the surfaces infected with T_3-phages were spread on the agar
plates which were covered with 0.1 ml of a bouillon culture from the corres-
ponding E. - coli strain.

Results and Discussion

   A. 1. In experiment groups II to IV and VI, VII (Table 1) I tested
the effect of PBL aerosols in varying agent concentrations for artificial
germ aerosols of staphylococci and coliphagens. During the pertinent empty
experiments, I atomized sterile tap water instead of the BPL solution. The
relative humidity in all experiments was rather constant and fluctuated only
very little around an average value of 70%, respectively, 50% (experimental
group VII). Table 1 shows the data for the series of experiments conducted
on the problem of room air disinfection, arranged by experimental groups I
to VII; every group of experiments consists of five, respectively, two indi-
vidual experiments. The measurement results N_a, N_b, N_c, N_d, N_e as well as
the computed survival rates (Q_b, Q_c, Q_d, Q_e)* and the disintegration rates
(K_b, K_c, K_d, K_e)** are shown in Table 2.

$$* \; Q_{[b, c, d, e]} = \frac{N_{[b, c, d, e]} \cdot 100}{N_a}$$

$$** \; K_{[b, c, d, e]} = \frac{\log N_a - \log N_{[b, c, d, e]}}{t}$$

   The formation of the survival and disintegration ratios made it pos-
sible to compare the measurement data also when the initial values were not
identical. I did not evaluate or reproduce the germ numbers determined with
the help of the sedimentation method because this method does not give us
comparable results and therefore does not seem suitable for a quantitative
determination. The average survival ratios from the empty experiments were
compared with those obtained in the disinfection experiments and the signi-

ficance of their difference was tested. Similarly, I compared two disinfection experiments, each time, with varying BPL concentration (Table 3). The statistical analysis was performed on the basis of equation (13)

$$s^2 x, x' = \frac{S(x^2) - \bar{x}S(x) + S(x'^2) - \bar{x}'S(x')}{N + N' - 2} \quad \text{(Common dispersion of samples)},$$

$$s_D = \frac{s^2 x, x'}{N} + \frac{s^2 x, x'}{N'} \quad \text{(Standard deviation of mean value difference)},$$

deviation $\quad t = \frac{\bar{x} - \bar{x}'}{s_D}$. The pertinent probability p was taken from the

probability table (13).

TABLE 1
SERIES OF EXPERIMENTS CONDUCTED

(1) Vers.- Gruppe	(2) Anzahl d. Versuche	(3) Testkeim	(4) Maßnahme	(5) Rel. Feuchte %	Temp. °C
I (Leer (6) versuche)	5	St. albus	Verneblung von sterilem Wasser 5 min lang (7)	70 ∓ 5	22 ∓ 2
II	5	St. albus	Verneblung von BPL (30%ig) 5 min lang (= ca. 0,44 mg Reinsubstanz/1 Luft) (8)	70 ∓ 5	22 ∓ 2
III	5	St. albus	Verneblung von BPL (30%ig) 1 min lang (ca. 0,21 mg Reinsubstanz/1 Luft) (9)	70 ∓ 5	22 ∓ 2
IV	5	St. albus	Verneblung von BPL (30%ig) 15 sec lang (= ca. 0,11 mg Reinsubstanz/1 Luft) (10)	70 ∓ 5	22 ∓ 2
V (Leer- (6) versuche)	2	Coliphagen (T₃)	Verneblung von sterilem Wasser 5 min lang (7)	70 ∓ 5	22 ∓ 2
VI	2	Coliphagen (T₃)	Verneblung von BPL (30%ig) 5 min lang (= ca. 0,5 mg Reinsubstanz/1 Luft) (11)	70 ∓ 5	22 ∓ 2
VII	2	Coliphagen (T₃)	Verneblung von BPL (30%ig) 5 min lang (= ca. 0,5 mg Reinsubstanz/1 Luft) (11)	50 ∓ 5	22 ∓ 5

Legend: 1--Group of experiments; 2-- Number of experiments; 3--Test germ; 4-- Measures taken; 5--Relative humidity %; 6--(Empty experiments); 7--Atomization of sterile water for 5 minutes; 8--Atomization of BPL (30%) for 5 minutes (--about 0.44 mg pure substance/1 air); 9--Atomization of BPL (30%) for 1 minute (--about 0.21 mg pure substance/1 air); 10--Atomization of BPL (30%) for 15 seconds (--approximately 0.11 mg pure substance/1 air); 11--Atomization of BPL (30%) for 5 minutes (--about 0.5 mg pure substance/1 air).

TABLE 2
MEASUREMENT DATA

(1) Versuchs- gruppe	N_a	N_b	N_c	N_d	N_e	Q_b	Q_c	Q_d	Q_e	K_b	K_c	K_d	K_e
I	2580	757	549	229	104	29,4	21,3	8,9	4,0	0,0266	0,0192	0,0210	0,0215
	2270	1063	1072	758	572	46,8	47,0	33,4	25,2	0,0165	0,0093	0,0095	0,0092
	1485	587	344	263	160	39,5	23,2	17,7	10,8	0,0202	0,0181	0,0150	0,0149
	1109	569	586	266	178	51,3	52,0	24,0	16,0	0,0145	0,0079	0,0124	0,0122
	756	361	215	175	183	47,8	38,5	23,1	24,2	0,0161	0,0156	0,0127	0,0095
x̄	1640	667	553	338	239	43,0	36,4	21,4	16,0	0,0188	0,0140	0,0141	0,0135
II	4258	25	16	32		0,58	0,37	0,75		0,1116	0,0692	0,0424	
	3556	13	12	27		0,38	0,35	0,76		0,1218	0,0762	0,0424	
	6219	45	33	36		0,72	0,53	0,57		0,1069	0,0650	0,0247	
	847	1	2	2		0,12	0,24	0,24		0,1464	0,0751	0,0525	
	3419	6	9	4		0,17	0,26	0,12		0,1377	0,0737	0,0586	
x̄	3660	18	14	20		0,39	0,35	0,49		0,1249	0,0718	0,0441	
III	1957	18	2	1		0,92	0,10	0,05		0,1018	0,0854	0,0658	
	2840	2	2	4		0,07	0,07	0,14		0,1576	0,0901	0,0570	
	2456	31	1	4		1,26	0,04	0,16		0,0949	0,0969	0,0558	
	2205	114	25	22		5,17	1,13	1,0		0,0643	0,0556	0,0400	
	1839	63	15	14		3,42	0,82	0,76		0,0732	0,0598	0,0424	
x̄	2259	46	9	9		2,17	0,43	0,42		0,0984	0,0776	0,0522	

Symbols: N -- number of germs(viruses) per liter of air; a,b,c,d,e - first, respectively, second, third, fourth, fifth measurement; Q - survival rate; K - disintegration rate; x - mean value.

Legend: 1--Experimental group.

TABLE 2 (contd)

(1) Versuchsgruppe	N_a	N_b	N_c	N_d	N_e	Q_b	Q_c	Q_d	Q_e			
IV	5740	1144	15	5		19,93	0,27	0,08		0,0350	0,0738	0,0612
	3064	42	3	6		1,37	0,10	0,19		0,0932	0,0859	0,0542
	695	47	12	4		6,75	2,02	0,57		0,0585	0,0504	0,0448
	3655	413	59	46		11,3	1,62	1,73		0,0473	0,0512	0,0380
	2820	244	92	18		8,67	3,26	0,63		0,0531	0,0425	0,0439
$\bar{x}$	3196	378	36	16		9,6	1,45	0,64		0,0574	0,0608	0,0484
V	1220	400	354	310	194	32,8	29,0	25,4	15,9			
	1966	730	422	404	319	37,6	21,5	20,5	16,2			
$\bar{x}$	1593	565	388	357	257	35,2	25,3	23,0	16,1			
VI	2731	0	0	0	0	0,0	0,0	0,0	0,0			
	1928	0	0	0	0	0,0	0,0	0,0	0,0			
$\bar{x}$	2329	0	0	0	0	0,0	0,0	0,0	0,0			
VII	2366	19	27	0	—	0,8	1,1	0,0	—			
	1080	34	12	2	0	3,1	1,1	0,2	0			
$\bar{x}$	1723	27	20	1		1,95	1,1	0,1				

Legend: (1) Experimental group.

Symbols: N—number of germs (viruses) per liter of air; a, b, c, d, e—— first, respectively, second, third, fourth, fifth measurement; Q—survival rate; K—disintegration rate; $\bar{x}$ —— mean value.

TABLE 3
RESULTS OF STATISTICAL ANALYSIS (t-test)

Vergleichene Versuchs-gruppen	Q_b			Q_c			Q_d		
	s_p	t	p	s_p	t	p	s_p	t	p
I— II	3,842	11,0905	<0,001	6,175	5,8380	<0,001	4,037	5,1795	<0,001
I—III	3,952	10,3314	<0,001	6,170	5,8298	<0,001	4,031	5,2046	<0,001
I—IV	4,231	7,8940	<0,001	6,201	5,6361	<0,001	4,046	5,1309	<0,001
II—III	0,2968	—5,9973	<0,001	0,2329	—0,3434	>0,05	0,2324	0,3012	>0,05
II—IV	1,7374	—5,3010	<0,001	0,5878	—1,8713	>0,05	0,3201	—0,4686	>0,05
III—IV	1,697	—4,3783	<0,01	0,6279	—1,6244	>0,05	0,3498	—0,6289	>0,05

Legend: Groups of experiments compared.

Symbols: Q_b, Q_c, Q_d—— survival rates in % of initial value (20 respectively 35 and 50 minutes after 1st measurement)

s_p——standard deviation of mean value difference

t ——deviation

p ——probability of deviation.

2. In the evaluation of the experiments we must keep in mind that the number of living viruses of an artificial virus aerosol will decrease even without treatment. In the empty experiment, the average survival rates for Staph. albus 20, 35, 50, respectively, 65 minutes after the first measurement was 43, 36.4, 21.4, respectively 16%; the rate for Coliphagens was 35.2, 25.3, 23.0, respectively, 16.1%.

3. After atomization of 0.44 mg BPL/l of room air the survival percentages for the staphylococci were below 0.5%. When the room concentration of BPL was about 0.21 mg/l, there were 2.17% surviving after 20 minutes, and less than 0.5% after 35 and 50 minutes. The corresponding survival rates at 0.11 mg BPL/l of air amounted to 9.6, 1.45, and 0.64%. The probability (p) that the difference in the mean values was accidental is always less than 0.001 (Table 3). In this connection we must consider that the time of action of the agent at the moment of the 2nd measurement is not 20 minutes but 15 minutes -- actually it is less than 15 minutes -- because the effective concentration of the disinfectant in the air could not have been achieved at the start of atomization. It was impossible to kill all of the staphylococci beyond 99.5% with BPL in the concentrations used. In this connection we might point out that the method used for determining the concentration of BPL in room air -- through conversion from the determination of the volume of the BPL solution sprayed -- undoubtedly involves a by no means inconsiderable spread of error. Experiments aimed at a continuing photometric concentration determination for BPL in room air have so far not led to any satisfactory results for methodological reasons so that I did not deem it advisable to reproduce these results here.

4. If we compare the disinfection experiments with each other, we do find a reliable difference (p = <0.01 respectively< 0.001) of the survival rates at the time of the 1st measurement after the atomization of BPL, although not during the further measurements.

TABLE 3

RESULTS OF STATISTICAL ANALYSIS (t-test)

Verglichene Versuchs-gruppen	Q_b			Q_c			Q_d		
	s_D	t	p	s_D	t	p	s_D	t	p
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I—III	3,952	10,3314	<0,001	6,170	5,8298	<0,001	4,031	5,2046	<0,001
I—IV	4,231	7,8940	<0,001	6,201	5,6361	<0,001	4,046	5,1309	<0,001
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Legend: Groups of experiments compared.

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4. If we compare the disinfection experiments with each other, we do find a reliable difference (p = <0.01 respectively< 0.001) of the survival rates at the time of the 1st measurement after the atomization of BPL, although not during the further measurements.

t_{90} VALUES AS EXPRESSION OF BACTERICILLING EFFECT OF BPL AEROSOL

(1) Versuchs-gruppe	(2) BPL-Konz. mg Reinsub-stanz/l luft	t_{90} (= 1/k)	Ct_{90}	k	K_b	(3) Signifikanzberechnung für k—K_b s_D	t	p
II	0,44	9,0	3,96	0,1111	0,1116			
	0,43	8,5	3,66	0,1176	0,1218			
	0,45	9,5	4,28	0,1053	0,1069			
	0,45	6,75	3,04	0,1482	0,1464			
	0,44	7,0	3,08	0,1429	0,1377			
$\bar{x}$	0,44	8,15	3,60	0,1250	0,1249			
						0,01148	0,00871	> 0,90
III	0,24	9,75	2,34	0,1026	0,1018			
	0,23	6,5	1,50	0,1538	0,1576			
	0,17	10,5	1,79	0,0952	0,0949			
	0,19	15,5	2,95	0,0645	0,0643			
	0,21	13,5	2,84	0,0741	0,0732			
$\bar{x}$	0,21	11,15	·2,28	0,0980	0,0984			
						0,02254	— 0,0177	> 0,90
IV	0,15	19,0	2,85	0,0526	0,0350			
	0,14	11,5	1,61	0,0869	0,0932			
	0,12	20,5	2,46	0,0488	0,0585			
	0,10	20,5	2,05	0,0488	0,0473			
	0,06	21,5	1,29	0,0465	0,0531			
$\bar{x}$	0,11	18,6	2,05	0,0567	0,0574			
						0,01240	— 0,05645	> 0,90

Legend: 1—Group of experiments; 2—BPL concentration, mg pure substance/l air;
3—Computation of significance when k - K_b.

Symbols: t_{90} -- Time in minutes required to kill 90% of the germs (viruses);
Ct_{90} -- Product of BPL concentration in mg/liter of air and value for t_{90};
k -- Value for decay rate obtained through conversion from equation $t_{90} = 1/k$;
K_b -- Disintegration ratio at time of 2nd measurement, calculated from

$$\frac{\log N_a - \log N_b}{t}$$

The fact that the survival ratios become increasingly similar as the duration of the action of the agent increases could not very well be an indication of the greater significance of the time factor for the bactericidal effect of the BPL aerosol (apart from the fact that this development is certainly dependent on the concentration). Instead, this appears to be explained by the below-threshhold dosing of the disinfectant, especially since it was impossible to kill all of the viruses 100% (experimental groups II, III, and IV). In future experiments it will therefore be necessary to increase the concentration.

5. In experimental groups VI and VII we checked the disinfectant

effect of a BPL aerosol, contain... ..5 ng agent/l of air, for an artificial virus aerosol of Coliphagens whic... ..d been used as model viruses in place of pathogenic animal viruses. As we can see in Table 2, it was impossible to prove the presence of phages afte... ...e first measurements following disinfection, when the relative humidity in the air was 70%. At 50% relative humidity there were very few viruses which survived, that is, we never had more than 3% surviving. The dependence of the disinfectant effect on the humidity in the air will have to be subject... to further detailed study. There will not be any need for a statistical analysis in this case and at this point.

(a) Graphische Ermittlung eines t_{90}-Wertes
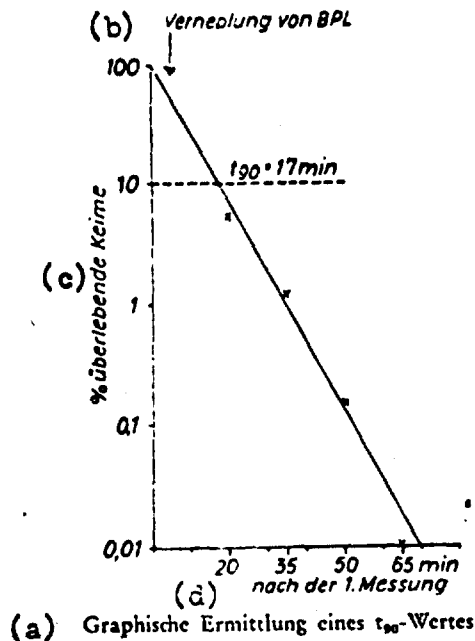
Legend: a--Graphic determination of a t_{90} value; b-- atomization of BPL; c--%of surviving viruses; d--after first measurement.

6. We can also compare the effectiveness of disinfectant aerosols if we determine the t_{90} values (Table 4), that is to say, the time required to kill 90% of the given viruses. The value for t_{90} according to Phillips (49), corresponds to the reciprocal value of the decay rate $(t_{90} = \frac{1}{k})$. This can be determined very easily in graphic form (see illustration); we simply plot the percentage of survival rates against the time in a coordinate system on semi-logarithmic paper; in typical cases the points plotted will lie along a straight line whose gradient will be equal to k. In general we get sufficiently precise t_{90} values if, in addition to the initial virus count (survival rate--100%), we also make a further virus number determination after disinfection. To the extent that the points in the coordinate system are not located along a straight line, I proceeded as described in order to determine the values given in Table 4. The fact that this does not give us any major errors is also borne out by a comparison of the graphically de-

termined t_{90} values through comparison of the decay rates obtained from the equation $t_{90} = \frac{...}{k}$ with the disinfection rates K computed according to

$$\frac{\log N_a - \log N_b}{t}$$ (Table 4) whose mean value differences are far outside the range of significance (p = $>$ 0.90). If we want to be able to compare the relative activities of various disinfection aerosols to each other, it is a good idea to determine the Ct_{90} values (Ct_{90} -- concentration of disinfecting aerosol in mg per liter of air multiplied by the time in minutes required to kill 90% of the viruses). The product formed from C and t_{90} in this case (with the exception of experimental group II) appears to be quite constant so that we can assume that there is a direct proportionality between the concentration and the disinfection effect. It will, however, take further experiments before we can make a final statement on this.

B. The results summarized in Table 5 shows that the BPL aerosol was able to kill staphylococci and T3 phages, adhering to the linoleum platelets, after 30 minutes while it was able to kill E. coli after 15 minutes. A similarly successful disinfection was reported by Hoffman and associates and by Dawson and associates with regard to the effect of the BPL aerosol (partly however involving higher concentrations) on germ carriers consisting of linen and paper which had been infected with pathogenic staphylococci, Subtilis spores, VEE yellow fever, smallpox, psittacosis viruses, respectively, Rickettsiae.

According to results available to date it appears that BPL, in aerosol form, would be a useful agent in the decontamination of rooms and perhaps even of entire buildings -- because of its good antimicrobial effect even at low temperatures. It is of course not a substitute for ethylene oxide gas which is known for its good germ-killing properties and penetrability even at low air humidity and whose applicability is found primarily in other areas.

TABLE 5
DISINFECTING EFFECT OF BPL AEROSOL ON SURFACES

Keimart (1)			15	(2) min-Einwirkzeit 30	45	60	120
Staph. albus	(3)	Versuch	(+)	ϕ	ϕ	ϕ	ϕ
	(4)	Kontrolle	+	+	+	+	+
SG 511		Versuch	(+)	ϕ	ϕ	ϕ	ϕ
		Kontrolle	+	+	+	+	+
E. coli		Versuch	ϕ	ϕ	ϕ	ϕ	
		Kontrolle	+	+	+	+	
Coliphagen (T₃)		Versuch	(+)	ϕ	ϕ	ϕ	ϕ
		Kontrolle	+	+	+	+	+

Legend: 1--Germ species; 2--Action in minutes; 3--Experiment; 4--Control.
Symbols: +   Heavy growth (bacteria patch (turf) respectively countless
                numbers of phage holes);

(τ) Weak growth (a few easily counted bacteria colonies, respectively, phage holes);

(φ) No growth.

Since BPL achieves the degree of effectiveness of formaldehyde gas after aerolization -- and since it exceeds this gas in many ways -- we might have some new possibilities here for more effective and more convenient room disinfection. A comparison of the other properties of formaldehyde with those of the BPL aerosol also clearly brings out the superiority of the latter: the very annoying persistence, the condensation along surfaces, the polymerization into paraformaldehyde and the required long period of ventilation after the application of formaldehyde are disadvantages which we need not anticipate when we use BPL aerosol. Here it is very important to note that BPL aerosol does not lead to the corrosion of metals and, according to past experience, does not attack other materials which are usually found in a room. We might furthermore mention that the agent is not combustible in concentrations which might exist under normal atmospheric conditions. We do not get a combusible mixture until we have 2 vol % in the air. However, the vapor pressure of BPL is such that, at 30° C, the concentration in the air along the saturation boundary is only about 0.6 vol% so that there is no danger of fire at all. In addition this concentration is considerably higher than the one required for disinfection.

So far we do not have much information on the toxicity of BPL aerosol. It becomes perceptible to the sense of smell at about 0.05 mg/l. A room concentration of more than 0.1 mg/l cannot be tolerated by man longer than 5 minutes because of the irritation to the tear ducts. Another disadvantage is the fact that best results with BPL can be achieved only at a relatively high humidity. But this shortcoming can be somewhat reduced since, in the production of the BPL aerosol from an aqueous solution, the humidity in the air can also be increased simultaneously.

It will take many investigations on the properties of BPL aerosol before we can arrive at a final judgement. According to past experience, however, we are justified in hoping that we can find a fully equivalent and even better substitute for formalin in the form of Beta-propiolactone.

Summary

This is the first report on investigations dealing with the disinfectant action of a Beta-propiolactone aerosol on artificial germ aerosols of staphylococci and Coliphagens used as model viruses. In addition we tested the disinfectant effect of the aerosol along surfaces. The article discusses the advantages and disadvantages of the aerolized substance, particularly in comparison to formalin, as well as its suitability in room disinfection.

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(Author's address: Dr. med. Karl-Heinz Husmann, Hygiene Institute, University of Mainz, Langenbeckstrasse 1.)