STUDIES ON MICROORGANISMS IN SIMULATED ROOM ENVIRONMENTS[1]

VII. Further Observations on the Survival Rates of Streptococci and Pneumococci in Daylight and Darkness[1]

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In an earlier report (Buchbinder, Solowey and Phelps, 1941) it was shown that both daylight and sunlight after passing through a window are lethal for streptococci maintained in a simulated room environment. Tests of the effect of diffuse daylight on one short chain strain of alpha hemolytic streptococcus yielded reproducible data which indicated a geometric death rate. Similar findings were obtained with a short chain strain of Group B beta hemolytic streptococcus. Tests on a long chain strain of Group A beta hemolytic streptococcus, however, yielded non-geometric death rates during the usual one and two hour test periods. It was calculated from the data that daylight would destroy 50 per cent of the very short chains of the alpha hemolytic strain in 45 minutes, whereas 200 minutes would be required for the same effect on the Group A strain. This divergence in death rates, as well as the non-geometric course of death for the Group A strain, were explained by the difference in average chain length of the two strains.

It was also found that the lethal power of diffuse daylight varied with its spectral distribution and intensity. Light from clear blue skies seemed to be most effective per foot-candle, whereas that from very cloudy skies was least effective. However, light from cloudy skies on all but the grayest days had the greatest overall killing effect, evidently because of increased intensity due to reflection from the clouds. Direct sunlight (through glass), as might be expected, was much more lethal than daylight, the median survival time[2] of the strain of alpha hemolytic strepto-

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[2] Median survival time is the time required under any given death rate for a diminution of the bacterial population to one-half its initial value. It is calcu-

coccus for sunlight being about five minutes. The killing power of sunlight per foot-candle apparently was not influenced by the intensity of the sun within the limits studied. Sunlight per foot-candle was inferior to diffuse daylight, probably because there is more blue light in diffuse daylight than in sunlight. It was also determined that the so-called daylight tubular fluorescent lamp had a unit killing power similar to that of daylight itself. It was considered unlikely, however, that the lamps as at present constituted have appreciable value as disinfecting agents because of their relatively low intensity.

In view of other findings (Buchbinder, Solowey and Solotorovsky, 1941; Buchbinder, Solotorovsky, Solowey and Ruhl-Koupal, 1941) which indicated that, in the absence of light, pathogenic streptococci can survive with practically undiminished virulence for considerable periods in indoor environment, these results suggested that solar energy in rooms might serve as an important deterrent to the spread of streptococcal and perhaps other infections.

The present paper reports a study of the effect of daylight on other strains of group A beta hemolytic streptococci and on several strains of pneumococci, in order to obtain comparative data on other strains of pathogenic cocci, and to inquire into the possibility that, because of unusual resistance to the deleterious effects of room environment, only certain strains of streptococci are capable of epidemic spread. Although there is no accepted criterion or even combination of criteria by which to differentiate epidemic from non-epidemic strains, it has been suggested by Keogh and his associates (1939) that the ability to resist destruction in air or dust may be one distinguishing characteristic of these epidemic types.

EXPERIMENTAL

Methods

The technics used were the same as those described in detail previously (Buchbinder and Phelps, 1941; Buchbinder, Solowey and Phelps, 1941). In essence, bacteria were sprayed into the air of a chamber and allowed to settle on filter paper in petri dishes. The dishes were then covered and exposed in a room for varying periods of time to daylight coming through a window.

lated from the geometric death rate which is identified by a velocity constant, k, the instantaneous or momentary rate of decrease.

After exposure the plates were poured with blood agar, incubated, and the colonies counted. The numbers were then compared with those of control plates sprayed at the same time but kept in the dark. The light intensity during each test was measured by a photometer and the character of the sky was recorded.

The strains of streptococci used, which were secured through the courtesy of Dr. Francis F. Schwentker, are all long-chain forms. They consist of given numbers of epidemic scarlatinal strains, isolated in several villages in Rumania, as well as non-epidemic strains which were obtained in one of these villages during an epidemic outbreak. Three strains of pneumococci were studied, one each of types I, II and III. They are all short chain forms, old laboratory strains of high rabbit virulence.

Results

In agreement with the finding previously reported on a single strain of Group A beta hemolytic streptococcus it was found in a number of instances that tests on other strains of this serological group, carried out simultaneously for one and two hours, yielded survival rates which were not geometric. The hourly survival rate was found to be much greater after one hour than after two hours. Such a result is probably due to the fact that a chain, when placed in a solid nutrient medium, will give rise to only one colony no matter how many cocci are in the chain, or conversely until the last individual coccus is dead. As might be expected tests carried out for longer periods yield more nearly geometric rates. For this reason most of the tests were run for two and four hour intervals, and all one hour survival rates which were found to be much greater than those of the parallel two hour test were discarded.

In table 1 a total of 56 tests on 12 epidemic and three non-epidemic strains of streptococci are listed. It is seen that the median survival time of the individual tests varied from one to eleven hours, and that no difference in susceptibility between the epidemic and non-epidemic strains is apparent. Nor are noteworthy differences observed when the findings for each strain are pooled; indeed even when the pooled result for all the epidemic and that for all the non-epidemic strains are compared, no statistically significant difference is found. Actually, the average median survival time of five hours for the non-epidemic strains is greater than that for the epidemic strains, which is four hours. This is the reverse of what would be expected if epidemic strains of

TABLE 1

The survival rates in daylight in simulated room environment of epidemic and non-epidemic strains of group A beta hemolytic streptococci

STRAIN NUMBER	HOURS OF EXPOSURE	AVERAGE NUMBER OF ORGANISMS		PERCENTAGE SURVIVAL	MEDIAN SURVIVAL TIME IN HOURS (CALCULATED)	MEDIAN SURVIVAL TIME PER STRAIN IN HOURS (CALCULATED)
		Control (dark)	Daylight			
Epidemic strains						
7199	1	429	394	92	6.5	
	2	371	260	70	3.9	
	1	389	334	86	4.6	
	2	371	251	68	3.6	
	1	54	47	87	5.0	
	2	43	36	84	7.9	
	1½	926	615	66	2.5	
	3	1157	601	52	3.2	
	4	64	30	48	3.7	4.5
7111	1	314	193	62	1.4	
	2	201	40	20	0.9	
	2	79	39	49	1.9	
	1½	569	493	86	6.9	
	3	559	392	69	5.6	
	2	320	256	80	6.2	
	4	317	207	65	6.5	
	2	215	143	66	3.4	
	4	203	60	28	2.2	3.9
6961	1	296	249	84	4.0	
	2	276	240	87	10.0	
	1	130	98	75	2.4	
	2	92	61	66	3.3	
	1½	268	215	80	4.7	
	3	306	179	58	4.8	
	4	46	23	50	4.0	4.6
6839	2	323	118	36	1.8	
	4	314	106	34	2.6	2.2
6853	2	164	95	58	2.6	
	4	188	81	43	3.3	3.0
6860	2	76	48	63	3.0	
	4	67	18	30	2.1	2.6
7074	2	273	191	70	3.9	
	4	284	96	34	2.6	3.3
7872	2	75	62	83	7.2	
	4	73	37	51	4.1	5.6
8199	2	237	137	58	2.5	
	4	249	98	39	3.0	2.8
7860	2	132	103	80	6.1	
	4	140	86	61	5.7	5.9
7865	2	136	105	77	5.4	
	4	130	47	36	2.7	4.1
7874	2	126	77	61	2.8	
	4	112	32	29	2.2	2.5
Average of all tests on epidemic strains						4.0

TABLE 1—*Concluded*

STRAIN NUMBER	HOURS OF EXPOSURE	AVERAGE NUMBER OF ORGANISMS		PERCENTAGE SURVIVAL	MEDIAN SURVIVAL TIME IN HOURS (CALCULATED)	MEDIAN SURVIVAL TIME PER STRAIN IN HOURS (CALCULATED)
		Control (dark)	Daylight			
Non-epidemic strains						
7486	2	118	69	58	2.6	
	2	223	150	67	3.5	
	4	199	109	55	4.6	3.6
7489	2	281	115	41	1.6	
	2	260	131	50	2.0	
	3	263	219	83	11.2	
	2	104	79	76	5.1	
	4	94	42	45	3.4	4.7
7483	2	171	146	82	7.1	
	2	64	51	80	6.2	
	3	523	326	63	4.6	
	2	74	60	81	6.6	
	4	65	41	63	6.0	6.1
Average of all tests on non-epidemic strains						5.0
Average of all tests						4.2

The epidemic strains were isolated in the following Rumanian villages: Heci (7199, 7111, 6961); Tatarusi (6839, 6853, 6860, 7074); and Hilita (7872, 8199, 7860, 7865, 7874). The non-epidemic strains were all isolated in the village of Hilita.

According to Schwentker (personal communication) all epidemic strains belong to type 10 whereas the non-epidemic strains belong to type 4 (7486 and 7483) and type 25 (7489).

Each daylight and control test was carried out with an equal number of petri plates, usually eight but sometimes seven or six.

streptococci depended for their ability to spread from person to person upon an enhanced resistance to the deleterious effects of room environment.

The survival rates in the dark of most of the Group A strains were also studied. Each test on each strain was run for one and two days, the two trials being started simultaneously. The results of 28 tests on the epidemic and non-epidemic strains are listed in table 2. Wide variations in the results of individual tests were again observed. The data can be separated arbitrarily into two groups, one of which contains 22 of the 28 trials and has a median survival time which ranges from 1.4 to 4.3 days, with a mean of 2.7, while the other combines the remaining six tests, which have extremes of 7.9 and 12.0 and a mean of 9.9 days. The difference between the means of these two groups is statistically significant. The group of long survival time, which comprises

TABLE 2

The survival rates in darkness in simulated room environment of epidemic and non-epidemic strains of group A beta hemolytic streptococci

STRAIN NUMBER	DAYS OF EXPOSURE	AVERAGE NUMBER OF ORGANISMS		PERCENTAGE SURVIVAL	MEDIAN SURVIVAL TIME IN DAYS (CALCULATED)
		Control (0 hour)	Dark		
7199	1	954	645	68	1.8
		52	43	83	3.6
7111	1	294	220	75	2.4
	3	294	174	59	4.0
	1	203	170	84	3.9
	2	190	169	89	11.9
6961	1	49	41	84	3.9
	2	53	45	85	8.5
6839	1	253	154	61	1.4
	2	262	158	60	2.8
6853	1	143	135	94	12.0
	2	124	86	69	3.8
6860	1	88	56	64	1.5
	2	74	47	64	3.1
7074	1	240	186	77	2.7
	2	271	184	68	3.6
7872	2	67	58	84	7.9
8199	1	252	207	82	3.5
	2	263	134	51	2.1
7860	2	136	118	87	9.7
7865	1	135	85	63	2.0
7874	1	107	74	69	1.9
	2	105	65	62	2.9
7489	1	73	68	93	9.7
	2	69	47	68	4.1
7483	1	637	422	66	1.7
	1	93	56	60	1.4
	2	83	36	43	1.6
Average of 22 tests ranging between 1.4 and 4.3.....					2.7 ± 0.46
Average of 6 tests ranging between 7.9 and 12.0.....					9.9 ± 0.82

Each dark and control test was carried out with an equal number of petri plates, usually eight, occasionally seven.

about one-fifth of the tests, may be regarded as aberrant although there is no ready explanation for its occurrence. No obvious differences in susceptibility in darkness is discernible between the epidemic and non-epidemic strains.

TABLE 3

The survival rates in daylight and darkness in simulated room environment of three strains of pneumococci belonging to types I, II, and III

STRAIN OF TYPE	HOURS OF EXPOSURE	DAYLIGHT				DAYS OF EXPOSURE	DARK			
		Average number of organisms		Percentage survival	Median survival time in minutes (calculated)		Average number of organisms		Percentage survival	Median survival time in hours (calculated)
		Control (dark)	Daylight				Control (0 hour)	Dark		
I	1	448	186	41	47	1	310	87	28	13.0
	2	386	24	6	28	2	323	58	18	19.5
	1	367	67	18	25	1	322	29	9	7.0
	2	312	5	2	20	2	344	18	5	11.3
	1	41	11	26	31	1	52	1	3	4.6
	2	41	2	4	25	2	53	1	2	8.6
				Average	29	1	426	52	12	7.9
						2	378	36	10	14.2
									Average	10.8
II	2	74	20	27	64	2	83	27	33	29.8
	4	107	3	3	46	1	218	18	8	6.7
	1	259	132	51	62	2	265	4	2	7.9
	2	184	52	28	66	1	117	4	3	4.8
	1	181	72	40	45				Average	12.3
	2	146	24	17	46					
				Average	55					
III	2	1291	126	10	34	1	1196	349	29	13.2
	4	1454	18	1	38	2	1063	102	10	14.2
	1	1270	357	28	33	1	1338	329	25	11.8
	2	1356	138	10	37	2	1275	78	6	12.0
	1	866	427	49	59	1	992	283	29	13.2
	2	803	173	22	54	2	861	93	11	14.9
				Average	42				Average	13.2

Each daylight and control test, and dark and control test was carried out with an equal number of plates, usually eight, occasionally less.

The survival rates in daylight and darkness of the three strains of pneumococci are given in table 3. There are three points of major interest to be noted in the table. The first is that the average mean survival times for all tests carried on in daylight (42 minutes) and in darkness (12 hours) are much shorter than those just noted for the Group A streptococci. The second point is that, unlike what was found for the Group A streptococci, the

daylight survival rates of the pneumococci are approximately geometric, as is shown by comparison of the paired one and two hour exposures. Finally, the three types of pneumococci do not seem to be significantly different among themselves in their resistance to daylight or dark.

DISCUSSION

It had been indicated previously that upon exposure to the action of daylight in simulated room environments for one and two hour periods, a streptococcus with chains short enough so that cocci which survived did not conceal the death of others, yielded survival rates which tended to be geometric. The only long chain strain studied earlier did not yield a geometric death rate after exposure for similar periods. The survival rates of this strain after one hour were much greater than that after two hours. It was shown that such a difference would be expected if equal susceptibility of individual cells was postulated and a uniform death rate was applied to chains of different length.

These observations are confirmed in the present paper. The many tests carried out on strains of long chain Group A beta hemolytic streptococci yielded results of the same nature as those previously described, for a single strain of this type. Tests carried out for one and two hours did not give geometric rates but such rates were obtained when exposures were made for periods of two and four hours. Likewise the median survival time of the 15 strains of Group A beta hemolytic streptococcus studied was about four hours, which is similar to that found previously for a single long chain strain. The findings with the pneumococci are also in accord with previous observations. These short chain forms yielded geometric death rates just as did the short chain strain of alpha hemolytic streptococcus which was studied earlier. The average median survival time for the pneumococci was about 44 minutes, as compared with 42 minutes for the alpha hemolytic streptococcus strain.

In view of the estimate in the previous report, which indicated a similar death rate per single coccus for long chain beta hemolytic and short chain alpha hemolytic streptococci, this similarity of the data for a larger group of strains of streptococci and for the strains of pneumococci points to a general homogeneity within the whole group. Similarity of the epidemic and non-epidemic strains of Group A beta hemolytic streptococci consequently does not seem remarkable. Variation in susceptibility to the deleterious effects of room environment is thus probably not a dis-

tinguishing feature of epidemic and non-epidemic strains of streptococci. This conclusion is strengthened by the data which suggest no difference in the death rates of the two groups of strains exposed to simulated room environment in the absence of light.

In contrast with the homogeneity of the daylight rates the dark death rates of short-chain species which includes strains studied here, as well as those reported on earlier, are very dissimilar. A strain of alpha hemolytic streptococcus, one strain of Group B beta hemolytic streptococcus and several strains of pneumococci which all had a similar high death rate in daylight, yielded average median survival times in the dark of 26, 132 and 12 hours, respectively.

Although it would seem from the differences in chain length that daylight on the whole may be more rapidly lethal to pneumococci in normal room environments than to the streptococci which cause human disease, this does not necessarily suggest that pneumococci cannot spread by the air-borne route. It is of interest that almost fifty years ago several workers (Bordoni-Uffreduzzi, 1891; Cassedebat, 1895; Ottolenghi, 1899) found that pneumococci in dried sputum exposed to diffuse daylight remained viable and retained their virulence for animals for extended periods of time, a fact which has been confirmed more recently by Stillman (1938). In 1905 Wood obtained similar findings and was led to consider the importance of air-borne transmission of pneumococcal infections. Nevertheless, because of his failure to recover pneumococci for more than an hour after spraying sputum in air, and since he found that dried and powdered sputum failed to yield viable organisms after one to four hours in the dark or one hour in sunlight, he came to the conclusion that "the risk of infection is largely confined to those in direct contact with the person whose excreta contains the organism". However, in 1917 Stillman presented evidence which suggests the possible clinical importance of air-borne infection by this organism and thus tends to negate Wood's conclusion. He was able to recover the infecting type of pneumococcus from the floors of homes of 26 of 52 patients with pneumonia and from the throats of family contacts of 15 of them at the time the patients were admitted to a hospital. The latter part of this observation has been repeatedly confirmed.

SUMMARY AND CONCLUSIONS

This report confirms and extends previous findings on the susceptibility to the bactericidal action of daylight of species of gram-

positive chain cocci maintained in a simulated room environment. As was suggested earlier the individual cocci in the chain die geometrically and are approximately equally susceptible to daylight regardless of species. These observations first indicated for one strain each of alpha hemolytic streptococci, Group B beta hemolytic streptococci and Group A beta hemolytic streptococci have now been demonstrated for 15 strains of the last species and for three strains of pneumococci. Differences in survival rates are apparently due largely to variations in average chain length. The larger chains are more resistant to daylight because a greater number of cocci per chain must be destroyed before the chain fails to generate a colony.

No significant differences in susceptibility to daylight between epidemic and non-epidemic strains of Group A beta hemolytic streptococci were found. This finding fails to support a suggestion of Keogh that ability to resist the destructive effects of room environment may be a distinguishing feature of epidemic strains.

Evidence is discussed that pneumococci, although more quickly destroyed by light because of their shorter chains than are beta hemolytic streptococci, may nevertheless be susceptible to airborne transmission.

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