THE INFLUENCE OF ATMOSPHERIC DRYING ON THE SURVIVAL OF WOUND FLORA

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(With 1 Figure in the Text)

The sequence of changes that take place in the death-rate of bacteria before, during and after the evaporation of their suspending fluids under normal atmospheric conditions has not received much attention. Dry environments are known to favour the survival of *Streptococcus pyogenes* and other Gram-positive cocci on blotting paper, in dried films and in dust (Hellat, 1948; Lidwell & Lowbury, 1950*a*, *b*). Coliform bacilli, on the other hand, though able to survive for long periods when dried *in vacuo* (see Morton & Pulaski, 1938), will tolerate a moist environment more readily than a dry one (Heller, 1941; Bardsley, 1948; Hellat, 1948), and are known to be more sensitive to the influence of drying on the skin than staphylococci or micrococci (Payne, 1949; Ricketts, Squire & Topley, 1951). The behaviour of bacteria during evaporation of airborne suspensions, and the conditions under which dissolved electrolytes exercise their greatest bactericidal action in such suspensions, have been studied by Dunklin & Puck (1948).

Differences of viability under dry and moist conditions are important in determining the environmental reservoirs of bacteria and the routes by which infections are transferred. They also have a bearing on the relation of humidity to infection in wounds; for example, in studying the exposure method of treating burns (Wallace, 1949) it would be useful to know what proportion of streptococci, staphylococci and coliform bacilli might be expected to survive in dried exudate.

In this paper we describe experiments on the survival of commoner wound and skin bacteria on glass cover-slips exposed, while drying and after, to an indoor atmosphere. We have assessed the proportion of survivors after drying not only from distilled water, but also from saline, serum and oleic acid, because of the possible influence of serum electrolytes, serous exudate and skin unsaturated fatty acids respectively on the fate of bacteria derived from wounds.

MATERIALS AND METHODS

Bacterial strains used

Of the commoner wound flora, we used fifteen strains of *Strep. pyogenes* (including types 4, 5, 10, 11, 12, 25 and 28), twenty-one strains of *Staphylococcus aureus* (including bacteriophage types 3*a*, 3*c*, 6, 7, 29, 31, 31*b*, 42*b*, 42*c*, 44, 47, 51, 52 and 55), and fifteen strains of *Pseudomonas pyocyanea*, some isolated from burns and other sources at the Birmingham Accident Hospital, and others kindly supplied to us by colleagues in England and Australia (including five serological types from Dr R. Christie of Melbourne). Twenty-two strains of micrococci were studied, twelve isolated from burns and ten from healthy skin that had been occluded from outside contamination for 2 days ('skin resident micrococci').

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Preparation of suspensions

Hedley Wright broth cultures of the bacteria after 24 hr. incubation were centrifuged at 2000 r.p.m. for ½ hr., washed with glass-distilled water and resuspended immediately before the test in one or more of the following menstrua: (1) horse serum, (2) sterile glass-distilled water, (3) sterile physiological saline, and (4) a freshly prepared aqueous solution of sodium oleate (equivalent to 0·1 mg. oleic acid per ml.).

Exposure to drying

Sterile grease-free cover-slips (⅝ × ⅝ in.) were mounted in pairs on glass slides. Approximately 0·02 ml. amounts of the freshly made bacterial suspensions were dropped on the cover-slips from standard dropping pipettes, and the drops were spread with a sterile platinum wire over the whole area of the cover-slip. They were then placed in cardboard boxes with loosely fitting lids to protect them from daylight, and left to dry. Films appeared to be dry in 30–60 min.

Enough cover-slips were prepared to allow extraction of two (or sometimes four) cover-slips of each suspension after one or, when required, two intervals of exposure, and for a control extraction of undried suspension. In all the experiments the control cover-slips were sampled immediately. Dried suspensions were sampled after approximately 100 min. and in some experiments after 2–3 days.

Extraction of cover-slips and bacterial counts

Cover-slips with dried films of bacterial suspension and the control cover-slips with the freshly applied bacterial suspensions were transferred with forceps to 1 oz. screw-cap bottles (universal containers) containing glass beads and 10 ml. amounts of broth saline. The lids were screwed on firmly, and the bottles were shaken by hand for 1 min. 1 in 10, 1 in 100 and 1 in 1000 dilutions of the extract were then made in broth saline. Four (exceptionally six) 0·02 ml. amounts of each dilution and the undiluted extracts were dropped from standard dropping pipettes on to plates of horse-blood agar. After incubation at 37° C. for 24 hr., the numbers of colonies growing from each drop were counted.

5 ml. amounts of broth were then added to the bottles, which were incubated at 37° C. for 24 hr. and subcultured to blood-agar plates for the detection of small numbers of bacteria.

RESULTS

With very few exceptions there was satisfactory agreement between the viable counts from replicate cover-slips. These were made at the best dilutions for counting, and the averages of the drop counts from two cover-slips were multiplied by the dilution factor, all results being expressed as the number of viable bacteria in 1 drop (0·02 ml.) of the undiluted extract. The proportions of bacteria surviving the periods of exposure were calculated as percentages of the counts obtained from the undried controls; these data are shown in Tables 1, 2, 3 and 4.

To assess the statistical significance of the differences in percentage survival of different organisms in several suspending fluids, each percentage has been counted as an attribute. When comparing the survival of different organisms in a single suspending fluid we have used the standard error of the difference of the means. When comparing the survival of a single organism in a variety of suspending fluids we have slightly reduced the variance by pairing the results from the same bacterial culture suspended on the same day in two or more different suspending fluids; and for this reason we have used the standard error of the mean of differences as an estimate of error. The numbers of organisms and suspending fluids tested on one day were limited by the equipment available, so that it was necessary for the experiment to cover a number of different days. The justification for ascribing significant differences in survival to species differences or to differences in suspending fluids rather than to other possible variables lies in the fact that each day two or more different species were tested in parallel and the same bacterial culture was tested in different suspending fluids. In this way a random distribution of the uncontrolled factors, such as humidity, was attempted, but it is not possible to estimate the effect of these factors on the final scatter of results.

Table 1. *Survival of* Staphylococcus aureus *on cover-slips*

	Viable counts per drop before and after drying of suspensions																	
	Before drying				After drying (100 minutes)								After drying (2–3 days)					
					In horse serum		In water		In normal saline		In 0·1 mg./ml. oleic acid		In horse serum		In water		In normal saline		In 0·1 mg./ml. oleic acid	
Strain	In horse serum	In water	In normal saline	In 0·1 mg./ml. oleic acid	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
B1331	2,400	1,800	2,900	1,800	3,000	127	2,500	140	1,800	62	290	16	2,200	92	290	16	380	13	32	1·8
PS47	5,500	5,350	9,600	7,000	6,850	125	5,150	96	3,750	39	770	11	3,200	58	375	7	625	6·5	130	1·8
PS3C	5,000	10,000	7,500	725	6,100	122	8,750	87	3,500	47	17·5	2·4	3,600	72	230	2·3	580	7·7	3·2	0·4
PS3*a*	23,000	22,500	—	9,900	8,800	38	1,500	6·6	—	—	690	7·0	3,000	13	650	2·9	—	—	4·0	0·04
PS29	11,000	16,000	—	10,000	6,000	55	800	5·0	—	—	500	5·0	100	0·9	800	5·0	—	—	0	0
PS6	20,000	19,000	—	17,000	11,000	55	10,000	53	—	—	700	4·1	100	0·5	300	1·6	—	—	2·0	0·01
PS55	—	40,000	33,000	17,000	—	—	20,000	50	8,000	24	15,000	88	—	—	—	—	—	—	—	—
PS52	—	28,000	25,000	14,000	—	—	2,100	7·5	860	3·4	4,000	29	—	—	—	—	—	—	—	—
PS7	—	27,000	31,000	32,000	—	—	12,000	44	23,000	74	1,700	5·3	—	—	—	—	—	—	—	—
PS31	—	30,000	33,000	24,000	—	—	17,000	57	3,200	9·7	320	1·3	—	—	—	—	—	—	—	—
PS42*b*	—	26,000	27,000	19,000	—	—	2,500	9·6	2,000	7·4	280	1·5	—	—	—	—	—	—	—	—
PS42*c*	—	—	37,000	—	—	—	—	—	10,000	27	—	—	—	—	—	—	—	—	—	—
PS44	—	—	8,000	—	—	—	—	—	350	4·4	—	—	—	—	—	—	—	—	—	—
PS51	—	40,000	39,000	29,000	—	—	25,000	62	40,000	103	16,000	55	—	—	—	—	—	—	—	—
PS31*b*	—	—	26,000	—	—	—	—	—	7,000	27	—	—	—	—	—	—	—	—	—	—
PS29 (repeat)	12,000	15,000	—	9,000	6,000	50	800	5·3	—	—	45	0·5	90	0·75	800	5·3	—	—	0	0
Average percentage of count before drying:						81·9		47·9		35·7		17·4		38·9		5·7		9·1		0·58

Table 2. *Survival of micrococci on cover-slips*

Viable counts per drop before and after drying of suspensions

	Before drying			After drying (100 min.)						After drying (2–3 days)					
				In water		In normal saline		In 0·1 mg./ml. oleic acid		In water		In normal saline		In 0·1 mg./ml. oleic acid	
Strain	In water	In normal saline	In 0·1 mg./ml. oleic acid	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
1	4,600	—	—	2,700	59	—	—	—	—	680	15	—	—	—	—
2	2,000	—	2,050	1,500	75	—	—	1,400	68	940	47	—	—	960	47
3	2,900	—	3,300	1,400	48	—	—	1,000	30	2,300	79	—	—	1,000	30
4	390	—	385	215	55	—	—	56	14·5	39	10	—	—	11	2·9
3883*a*	22,000	27,000	15,000	2,500	11	2,500	9·2	2,300	15	—	—	—	—	—	—
3887	19,000	18,000	17,000	2,200	12	1,300	7·2	1,400	8·2	—	—	—	—	—	—
3891*b*	32,000	30,000	16,000	21,000	66	15,000	50	440	2·7	—	—	—	—	—	—
3891*c*	38,000	29,000	35,000	36,000	95	10,500	36	1,550	4·3	—	—	—	—	—	—
3888*e*	46,000	50,000	20,000	20,000	42	5,000	10	370	1·8	—	—	—	—	—	—
3891*a*	44,000	45,000	20,000	10,000	23	2,500	5·5	60	0·3	—	—	—	—	—	—
3892*b*	9,500	10,000	4,500	3,700	39	4,100	41	1,300	29	—	—	—	—	—	—
3883	—	36,000	31,000	—	—	23,000	64	12,000	39	—	—	—	—	—	—
3869	—	4,400	—	—	—	500	11	—	—	—	—	—	—	—	—
4251*b*	—	55,000	39,000	—	—	500	0·91	5,200	13	—	—	—	—	—	—
4237*b*	—	60,000	—	—	—	8,200	14	—	—	—	—	—	—	—	—
4239*b*	—	44,000	—	—	—	2,600	5·9	—	—	—	—	—	—	—	—
Average percentage of count before drying:					47·7		21·2		18·8		37·7				26·6

Table 3. *Survival of* Streptococcus pyogenes *on cover-slips*

	Viable counts per drop before and after drying of suspensions																			
	Before drying				After drying (100 min.)								After drying (2–3 days)							
Strain and type	In horse serum	In water	In normal saline	In 0·1 g./ml. oleic acid	In horse serum		In water		In normal saline		In 0·1 mg./ml. oleic acid		In horse serum		In water		In normal saline		In 0·1 mg./ml. oleic acid	
					Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
962(4)	400	775	775	—	875	219	490	63	900	116	—	—	450	112·5	330	43	390	50	—	—
455(11)	1,800	1,600	1,050	3·5	2,200	122	460	29	140	13	0	0	1,700	94	160	10	140	13	0	0
1970(28)	10,000	1,600	—	—	7,600	76	1,600	100	—	—	—	—	5,400	54	1,600	100	—	—	—	—
Stock(5)	8,600	9,900	—	—	3,900	45	1,700	17	—	—	—	—	1,100	13	87	0·88	—	—	—	—
3(25)	7,400	7,700	—	—	4,700	64	3,300	43	—	—	—	—	1,200	16	730	9·5	—	—	—	—
2814(5)	9,000	10,000	—	—	4,000	44	1,700	17	—	—	—	—	1,100	12	86	0·86	—	—	—	—
1156(12)	1,800	1,700	—	—	900	50	160	9·4	—	—	—	—	130	7·2	40	2·4	—	—	—	—
Stock(4)	—	—	—	440	—	—	—	—	—	—	0·5	0·11	—	—	—	—	—	—	—	—
Stock(10)	—	—	—	14	—	—	—	—	—	—	0·25	1·8	—	—	—	—	—	—	—	—
Stock(11)	—	—	—	80	—	—	—	—	—	—	0·25	0·31	—	—	—	—	—	—	—	—
Stock(3)	—	—	—	0	—	—	—	—	—	—	0	0	—	—	—	—	—	—	—	—
Stock(12)	—	—	—	0	—	—	—	—	—	—	0	0	—	—	—	—	—	—	—	—
Stock(14)	—	—	—	0	—	—	—	—	—	—	0	0	—	—	—	—	—	—	—	—
Stock(5)	—	—	—	0	—	—	—	—	—	—	0	0	—	—	—	—	—	—	—	—
1552(25)	650	2,970	2,100	1·25	98	15	130	4·4	40	1·9	0	0	42	6·5	16	0·54	19	0·9	0	0
Average percentage of count before drying:						79·4		35·3		43·6		0·74		39·4		20·9		21·3		0

Table 4. *Survival of* Pseudomonas pyocyanea *on cover-slips*

	Viable counts per drop before and after drying of suspensions																			
	Before drying				After drying (100 min.)								After drying (2–3 days)							
				In 0·1 mg./ml.	In horse serum		In water		In normal saline		In 0·1 mg./ml. oleic acid		In horse serum		In water		In normal saline		In 0·1 mg./ml. oleic acid	
Strain	In horse serum	In water	In normal saline	oleic acid	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
589	2,200	4,600	—	7,400	300	14	820	18	—	—	205	2·8	120	5·5	71	1·5	—	—	2·5	0·034
'*x*'	3,400	19,500	—	18,500	45	1·3	156	0·8	—	—	6	0·032	0	0	0*	0*	—	—	0*	0*
Watts	—	7,600	—	16,000		—	380	5·0	—	—	1·1	0·007	—	—	24	0·32	—	—	0	0
P 8	2,000	4,100	5,250	—	87·5	4·4	11	0·27	1·4	0·027	—	—	1·6	0·08	0	0	0*	0*	—	—
B 1212	1,600	4,750	4,750	—	360	23	88	1·8	0·87	0·018	—	—	1·0	0·062	0*	0*	0·08	0·002	—	—
MB 1040	1,750	7,850	6,250	—	375	21	475	6·1	0·25	0·004	—	—	1·9	0·11	0·08	0·001	0·08	0·001	—	—
B 1387	1,300	9,900	7,000	6,600	76	5·8	110	1·1	13	0·19	1·25	0·019	1·5	0·12	0*	0*	0*	0*	0	0
P 15	10,000	21,000	—	9,000	2,000	20	3,000	14	—	—	3·5	0·039	0*	0*	0*	0*	—	—	0	0
P 36 (large colony)	—	14,000	12,000	6,000	—	—	600	4·3	400	3·3	3·5	0·058	—	—	—	—	—	—	—	—
P 36 (small colony)	—	12,000	16,000	4,000	—	—	700	5·8	1,700	11	14	0·35	—	—	—	—	—	—	—	—
Cardiff	—	—	3,500	1,200	—	—	—	—	0·5	0·014	0·025	0·0021	—	—	—	—	—	—	—	—
Ward	—	2,900	2,800	1,500	—	—	0·25	0·009	0*	0*	0·4	0·027	—	—	—	—	—	—	—	—
CTAB	—	14,000	18,000	13,000	—	—	100	0·71	270	1·5	10	0·077	—	—	—	—	—	—	—	—
1045	—	4,900	4,800	3,000	—	—	140	2·8	650	14	7	0·23	—	—	—	—	—	—	—	—
P 4	—	—	13,500	10,000	—	—	—	—	1,500	11	140	1·4	—	—	—	—	—	—	—	—
P 50	—	—	78,000	75,000	—	—	—	—	27,000	35	340	0·45	—	—	—	—	—	—	—	—
Average percentage of count before drying:						12·8		4·6		6·3		0·42	12·6	0·84		0·23		0·0007		0·007

*Growth occurred in fluid used for extracting cover-slips.

Survival of bacteria in aqueous suspensions

Three strains each of *Strep. pyogenes*, *Staph. aureus* and *Ps. pyocyanea*, and two strains of micrococci showed no significant drop in viable count after standing 100 min. in aqueous suspension.

Survival of bacteria dried from aqueous suspensions

The proportion of bacteria which survived the drying process was derived from the difference between viable counts of control undried cover-slips and of cover-slips allowed to stand for 100 min., i.e. slightly longer than the period required for evaporation of visible moisture (Tables 1–4).

Strep. pyogenes, *Staph. aureus* and micrococci behaved in a similar manner. In all three there was a sharp fall in the average viable count; different strains of a particular organism showed great variation in the proportion of survivors. The percentage of survivors varied from 4·4 to 140 % (means 35·3, 47·7, 47·9). With *Ps. pyocyanea*, however, the proportion of survivors was lower, varying from 0·009 to 18 % (mean 4·6). When the different organisms were compared with each other in terms of the average proportion of organisms surviving after 100 min., *Strep. pyogenes*, *Staph. aureus* and micrococci were all found to have significantly higher proportions of survivors than *Ps. pyocyanea* (*Strep. pyogenes* and *Ps. pyocyanea*: difference between means = 30·7, S.E. of difference = 8·9, $t = 3·4$, $P = < 0·01$; *Staph. aureus* and *Ps. pyocyanea*: difference between means = 43·3, S.E. of difference = 11·7, $t = 3·5$, $P = < 0·01$; *Micrococcus* and *Ps. pyocyanea*: difference between means = 43·1, S.E. of difference = 7·4, $t = 5·78$, $P = < 0·001$). There was no significant difference between the average proportions of *Strep. pyogenes*, *Staph. aureus* and micrococci surviving at 100 min.

Influence of the suspending medium

Serum was shown to have a significant protective action during the period of drying. When the three species tested (*Strep. pyogenes*, *Staph. aureus* and *Ps. pyocyanea*) were grouped together, the mean difference between proportions of survivors in the two menstrua was 26·3 (S.E. = 8·13, $t = 3·23$, $P < 0·01$). When species were taken singly, a significant difference between the proportions of survivors in serum and in water was found with *Staph. aureus* (mean difference = 25·6, S.E. = 8·64, $t = 2·98$, $P < 0·05$); with the other organisms the proportion of survivors in serum was higher than it was in water, but 5 % significance levels were not reached (*Ps. pyocyanea*: mean difference = 6·77, S.E. = 3·3, $t = 2·05$, $P > 0·05$; *Strep. pyogenes*: mean difference = 44·0, S.E. = 19·7, $t = 2·23$, $P > 0·05$).

Saline suspensions gave variable results, with average survival rates at 100 min. either slightly higher (*Strep. pyogenes* and *Ps. pyocyanea*) or lower (*Staph. aureus* and micrococci) than those of watery suspensions.

Oleic acid was used in these experiments because it is known to be a major constituent of skin unsaturated fatty acids. The strength used (0·1 mg./ml.) was one that had been shown in the test-tube (Ricketts *et al.* 1951) to have some bactericidal action on *Strep. pyogenes*, but to have no demonstrable effect on

Staph. aureus, skin resident micrococci or *Ps. pyocyanea*. It remained to be seen whether these organisms, suspended in such low concentrations of oleic acid, were influenced by it during drying. The results (Table 3) confirm that 0·1 mg./ml. oleic acid had a marked bactericidal action on streptococci. The new finding (Tables 1, 2 and 4) was that this strength of oleic acid during drying significantly increased the fall in viable counts of *Ps. pyocyanea* and micrococci as well as of *Staph. aureus*.

With *Ps. pyocyanea*, when suspensions of ten strains in water and in oleic acid solution were compared, the mean difference between proportions of survivors in the two menstrua was 4·88 (S.E. = 1·73, $t = 2{\cdot}92$, $P = 0{\cdot}02$). A similar comparison of ten strains of *Micrococcus* showed a similar difference between the proportions of bacteria surviving in the two menstrua (mean difference = 29·1, S.E. = 11·9, $t = 2{\cdot}46$, $P < 0{\cdot}05$). The difference between the proportions of thirteen strains of *Staph. aureus* surviving in oleic acid and in water was also significant (mean difference = 30·5, S.E. = 13·0, $t = 2{\cdot}34$, $P < 0{\cdot}05$).

That micrococci showed as great a sensitivity to oleic acid on drying as did *Staph. aureus* is of interest. As this finding contrasted with previous results (Ricketts *et al.* 1951) on *in vitro* sensitivity, we tested six strains of *Staph. aureus* and six strains of skin resident micrococci which had previously been shown to differ significantly in their resistance to 1–10 mg./ml. oleic acid in the test-tube. The effect of drying in the presence of 0·1 mg./ml. oleic acid was the same for each organism (*Staph. aureus*: average survival 2·5%; micrococci: average survival 2·6%). The contrast in the fate of these organisms when exposed to oleic acid in the test-tube and while drying on a cover-slip is discussed below.

Survival of bacteria in dried films

In sixty-four out of sixty-nine experiments, the viable count was lower at 2–3 days than at 100 min. Viable organisms were still present in fifty-eight experiments after 2–3 days, which demonstrates the persistence of some organisms after being dried on cover-slips under atmospheric conditions. Our preliminary results suggest that the sharp fall in viable count during the evaporation of the suspending fluid is followed in all the species studied by a much slower death-rate of the organisms which survive drying (see Fig. 1).

Relation of viability on drying to Gram-staining reaction

The studies reported here and elsewhere (Heller, 1941; Bardsley, 1948; Hellat, 1948; Payne 1949; Ricketts *et al.* 1951) suggest that Gram-negative bacilli are less viable under the influence of atmospheric drying than Gram-positive cocci. To test whether this difference in viability is a general phenomenon related to the Gram-staining reaction would necessitate an extensive study on many strains of different bacterial species. An approach to the problem was made in studying the viability of a strain of *Staph. aureus* (209) and of its Gram-negative mutant (*Micrococcus* P.T.) obtained by repeated subculture of strain 209 through increasing concentrations of penicillin (Bellamy & Klimek, 1948; Gale & Rodwell, 1948). We are indebted to Dr E. F. Gale for these strains.

Aqueous suspensions of these organisms were prepared in the manner described above, and their viability on drying was tested by exposure of films on cover-slips. Two coverslips from suspensions of three separate cultures of each strain were extracted immediately after preparation, and after exposure to the atmosphere for 100 min. and for 48 hr.

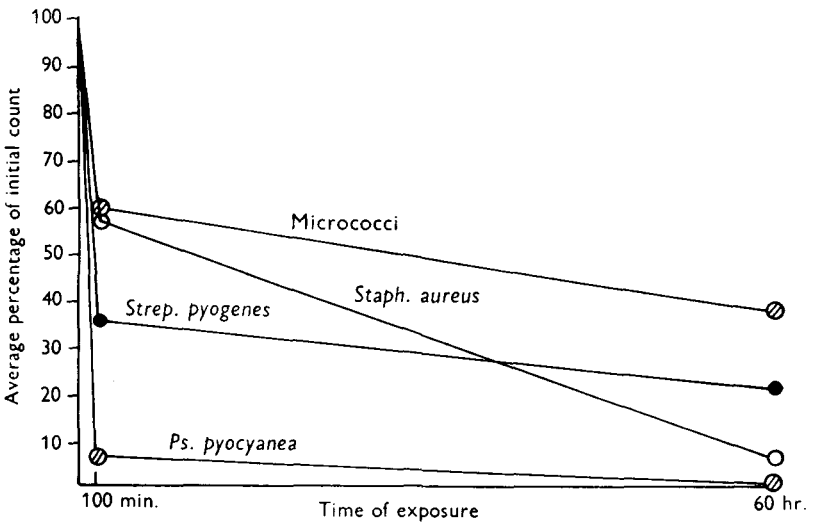

Fig. 1. The average percentage of survivors at 100 min. and approximately 60 hr. after exposing aqueous suspensions of seven strains of *Staph. aureus*, four strains of micrococci, eight strains of *Strep. pyogenes* and eight strains of *Ps. pyocyanea* on cover-slips in cardboard boxes. The films were all dry before the sampling at 100 min.

Table 5. *Survival of Gram-positive and Gram-negative* Staphylococcus aureus *on cover-slips*

		Viable count from average of two cover-slips			
		Strain 209 (Gram-positive)		*Micrococcus* PT (Gram-negative)	
Time of sampling	Suspension	Count	% of initial count	Count	% of initial count
Before drying (initial count)	1	13,800	—	5,600	—
	2	14,400	—	13,600	—
	3	13,800	—	7,600	—
After drying (100 min.)	1	1,380	10·0	1,020	17·2
	2	1,940	13·0	710	5·2
	3	1,100	7·9	760	10·0
After drying (48 hr.)	1	210	1·5	3·2	0·06
	2	133	0·9	0·1	0·0008
	3	33	0·2	16·4	0·2

Table 5 shows the average viable count of bacteria extracted from these cover-slips. It can be seen that there is no consistent difference in the proportion of Gram-positive and Gram-negative staphylococci surviving after 100 min.—that is, after the period of drying. In these experiments, therefore, it appears that the

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Gram-staining reaction is unrelated to the viability of the organisms on drying. The proportion of survivors after continued exposure of the dried films, however, was smaller among the mutants than among the original staphylococci.

DISCUSSION

In these experiments, drying of bacterial suspensions on cover-slips was associated with a sharp fall in viable counts of all organisms tested, the effect being much greater for *Ps. pyocyanea* than for the Gram-positive cocci. Serum slightly protected all the organisms during drying, while oleic acid reduced the proportion of survivors. These data are of interest in relation to the problems of self-sterilization of the skin, of wound sepsis, and of environmental hygiene.

Previous studies in this Unit on the elimination of bacteria from the skin (Ricketts *et al.* 1951) have shown that two factors, 'drying' and the action of unsaturated fatty acids, are involved. The effect of 'drying' was recorded on human skin sites on which the rate of evaporation was artificially varied; the effect of fatty acids was recorded *in vitro* in peptone-water suspensions not allowed to dry. These and earlier experiments (Burtenshaw, 1938, 1942) suggested that in a moist environment, skin unsaturated fatty acids were a major factor in the self-sterilizing action of the skin against *Strep. pyogenes* and, in smaller measure, also against *Staph. aureus*, but had little action on skin resident micrococci or *Ps. pyocyanea*. The added effect of 'drying' was more difficult to define. It was a major factor for *Ps. pyocyanea*, which persisted in a moist environment, and a possible factor for *Staph. aureus*.

The experiments described in the present paper define more clearly some of the mechanisms that may be involved in the 'drying' of bacteria on human skin. Drying caused a more rapid elimination of *Ps. pyocyanea* than of *Strep. pyogenes*, *Staph. aureus* and micrococci, but all of these organisms showed some reduction during the drying process, which varied considerably with the strain of the organism. Indeed, some strains of *Ps. pyocyanea* showed a higher proportion of survivors after drying than some strains of the Gram-positive cocci. Oleic acid showed, as expected, a vigorous action against *Strep. pyogenes* in drying films. In the previous studies, *Ps. pyocyanea* and skin-resident micrococci were found relatively resistant to 1–10 mg./ml. of oleic acid in a test-tube, a concentration that killed *Staph. aureus*. In the present experiments, however, *Ps. pyocyanea* and micrococci while drying on a cover-slip were shown to be at least as sensitive to 0·1 mg./ml. oleic acid as *Staph. aureus*.

Possible explanations of the differences between tests on a cover-slip and those in the test-tube are: (1) a greater susceptibility of *Ps. pyocyanea* and other organisms to various agencies during the process of drying; (2) the gradual saturation of the suspending fluid with oleic acid during evaporation of its solvent; and (3) the operation of other physico-chemical factors during drying such as changes in reaction. The similar sensitivity to 0·1 mg./ml. oleic acid during drying of staphylococci and micrococci, the former sensitive and the latter resistant to 1–10 mg./ml. oleic acid in a test-tube, suggests the operation of some additional and possibly non-specific factor during drying.

Applying these lessons to the problems of preventing wound sepsis, it might be argued that the retention of moisture under wound dressings will favour both the survival and the multiplication of colonizing organisms, particularly of *Ps. pyocyanea* and other coliform bacilli. This disadvantage is offset by the protection afforded to the wound and to the environment by dressings. However, in regions which cannot be adequately protected by dressings against infection from the environment (e.g. buttocks and face), the exposure of burns and perhaps of some other injuries to a dry atmosphere is not only convenient, but may lead to a reduction in the numbers of colonizing bacteria. The bactericidal effect of drying is significantly but not markedly reduced by serum.

Contamination of dust and, indirectly, of air by staphylococci and streptococci is well recognized (e.g. Brown & Allison, 1937; Lidwell & Lowbury, 1950*a*). Organisms more sensitive to drying, however, are unlikely to be found in large numbers in dust, and might be expected to spread by contact or fomites. Dust, however, must be considered as a possible vehicle of organisms relatively sensitive to drying (e.g. *Ps. pyocyanea*), in view of the regular survival of small numbers of organisms and the higher resistance of some strains to drying. Our results emphasize the fact that although drying reduces the number of bacteria it rarely eliminates them.

Our experiments on *Ps. pyocyanea* agree with those of Payne (1949) on *Bacterium coli* in finding a destruction of bacteria during evaporation of suspensions in distilled water, and which must therefore be distinct from the bactericidal effect produced by concentration of dissolved electrolytes. There is some evidence that dissolved sodium chloride may accelerate the death-rate of suspended organisms during the process of drying (Dunklin & Puck, 1948) but protect the organisms when they are dry (Heller, 1941). In our experiments, however, no consistent differences between saline and aqueous suspensions were observed.

Except when antibacterial substances were present in solution, the sharp fall in count in suspending fluids during the first 100 min. must be ascribed to the process of evaporation which occupied the first part of that period. The mechanism responsible for this effect, however, is obscure. It occurs with Gram-positive cocci and with Gram-negative bacilli, to a significantly greater extent in one species of the latter than in the former. It is suggested that this difference is probably unrelated to the Gram-staining reaction, since a Gram-negative *Staph. aureus* was shown to be as viable on drying as the Gram-positive organism from which it was derived by repeated subculture in the presence of increasing concentrations of penicillin. Numerous experiments with different Gram-positive and Gram-negative strains would be required for the confirmation of this view.

SUMMARY

Suspensions of *Strep. pyogenes*, *Staph. aureus*, *Ps. pyocyanea* and micrococci in serum, distilled water, saline and oleic acid solution were allowed to dry on cover-slips. The numbers of bacteria surviving after periods of exposure were estimated from viable counts of extracted cover-slips.

All the organisms tested showed after drying a fall in viable count that was absent in aqueous suspensions not allowed to dry. *Ps. pyocyanea* was shown to have a lower survival rate on drying than the other organisms.

Serum afforded moderate protection to the suspended organisms during the period of drying.

0·1 mg./ml. oleic acid caused a rapid destruction of *Strep. pyogenes* and a significant destruction of *Ps. pyocyanea* as well as of *Staph. aureus* and micrococci in drying films of these organisms. The similarity of the fate of *Staph. aureus* and micrococci during drying in low concentrations of oleic acid is in contrast to the previously reported greater sensitivity of *Staph. aureus* to higher concentrations of oleic acid (1–10 mg./ml.) in the test-tube.

The implications of these findings are discussed in relation to skin and wound flora, and to environmental hygiene.

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