associated with aseptic techniques rather than for the engineering methods applicable to the control of aerosols and dust.

Some rather heroic efforts have been made to control airborne infection in pediatric and surgical wards and operating rooms with controlled ventilation and ultraviolet irradiation. It is a safe generalization to say that, the more carefully controlled these experiments, the less impressive the evidence in favor of airborne infection has been. As emphasized by Dr. Williams, when strict asepsis is enforced to minimize contact and droplet infection, the rate of hospital-acquired infection is reduced to well below 5%, and may reach 1%. It is exceedingly difficult to prove whether this low residual rate results from airborne infection or from failure of the aseptic techniques to eliminate all contact.

Dr. Williams has drawn guarded conclusions regarding the role of airborne staphylococcal infection in hospitals. He has emphasized the multiple sources of infection and modes of spread. He admits that "we have insufficient precise evidence" on their relative importance. The epidemiological evidence, also far from precise and based on more general considerations, supports his caution.

Discussion

Viability of Hospital Staphylococci in Air

K. C. STRASTERS[1] AND K. C. WINKLER

Laboratory for Microbiology, State University, Utrecht, The Netherlands

The role of airborne transmission in the spread of staphylococci in hospitals (3) has not been definitively established. Though several authors studied the survival of staphylococci in air and on surfaces (1, 2, 4), exact knowledge of the survival of "epidemic" and "nonepidemic" strains is scarce.

The survival of a number of strains (for description, see Table 2) in air of differing relative humidity (RH) was studied by spraying with a direct spray (FK8) in a static system (4,000 liters) and sampling 10 liters in a slit sampler on blood-agar plates (Fig. 1a). The reference strain no. 1600 (isolated from a nose swab, phage type 187) showed a low decay rate at 50% RH and an increased decay rate at high RH (Fig. 2). This effect was generally found with most strains. During the experiments, it became clear, however, that by varying the growth medium, the age of the culture, the suspension medium, the method of aerosolization, and the composition of the collection plates, any desired result could be obtained. Decay curves could be logarithmical or curved, and the effect of relative humidity could be very marked or nonexistent. This variability was specially marked at high relative humidities, and less so below 50%. The late

[1] Deceased 25 October 1965.

TABLE 1. *Survival in air of strain isolated in hospitals compared with reference strain 1600 (90% RH)*[a]

Strain	Phage type	$K \times 10^{-4}$	K/K_{1600}
1600	187	87, 81, 79, 89, 85, 95, 82, 96, 85 95, 76, 97	1.00
1	80/81	95	1.09
7	80/81	74	0.94
17	80/81	77	0.81
18	52/80/81	73	0.77
3	52/80/81	77	0.89
8	52/80/81	70	0.89
1330 A	52/80/81	76	0.94
1451 A	52/80/81	70	0.79
2	NS I–III	84	0.97
1330 B	NS I–III	87	1.07
1451 B	NS I–III	76	0.89
4	III	79	0.98
10	III	102	1.15
11	III	92	1.03
16	III	108	1.14
15	83 A	114	1.27
13	III type A	117	1.38
9	II	79	1.00

[a] Three strains were tested daily together with the reference strain. K/K_{1600} was calculated with the K_{1600} observed on that day. Calculation based on the mean of K_{1600} reduced the differences.

appearance of small colonies on collection plates at high RH (Fig. 1b) indicated that many organisms were damaged and started growth only after a long lag.

An instance of variability with culture age is given in Fig. 3 and 4. Figure 3 gives the growth curve of strain 1600 in nutrient broth (Difco). At the indicated times (↑), the number of single organisms and of clumps of two, three, or more organisms was determined, or a sample of the

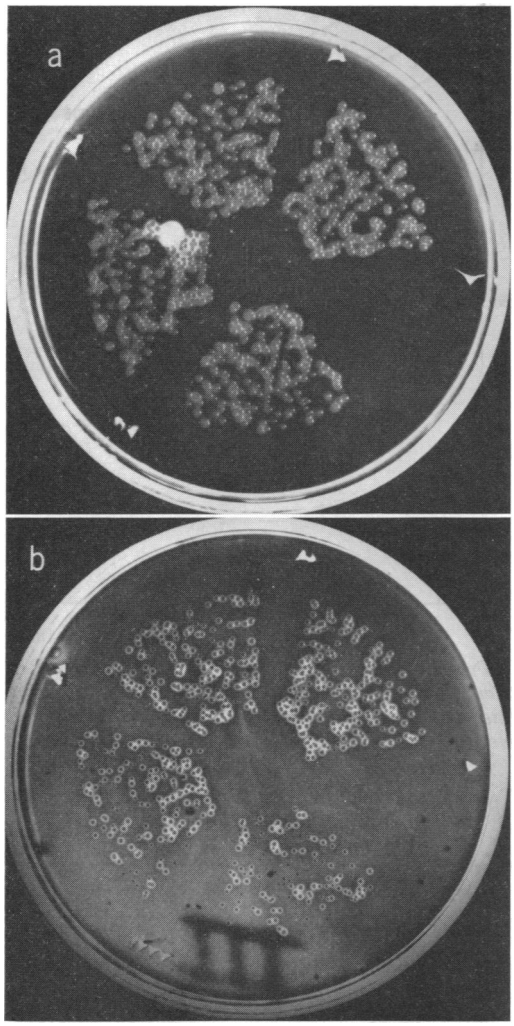

FIG. 1. *Colonies of staphylococci (strain 1600) recovered from aerosols in air of 20.0 C on blood-agar plates in a slit sampler. The four sectors represent 20-min (10-liter) samples 40, 45, 50, and 60 min after aerosolization; (a) 40% RH, uniform colonies; (b) 80% RH, increasing numbers of small colonies due to metabolic damage in later samples.*

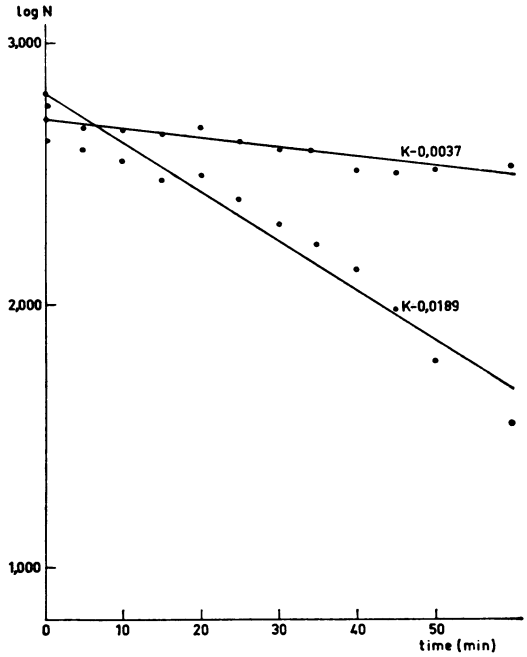

FIG. 2. *Decay curves of Staphylococcus no. 1600 at 39% RH (●) and at 75% RH (○).*

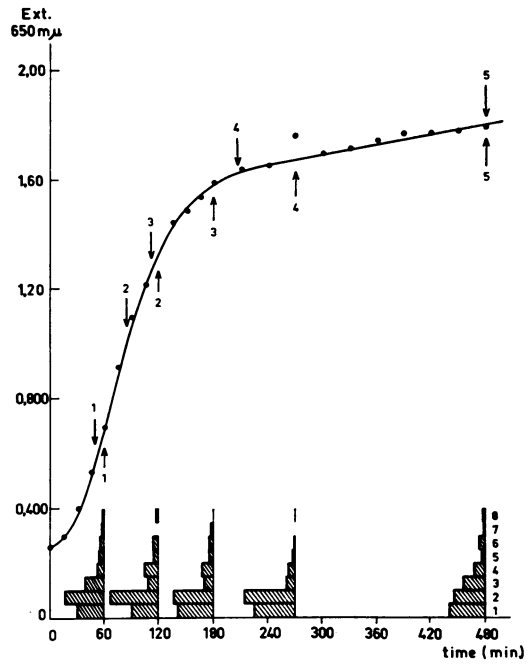

FIG. 3. *Growth curve of strain 1600, with frequency distribution of single organisms and clumps of various sizes shown at the bottom of the figure (right-hand scale). At the indicated times (↓), samples were diluted and aerosolized (compare with Fig. 4).*

culture was diluted (↓) and aerosolized. Figure 4 gives the corresponding decay curves at 70% RH.

Finally, we arrived at an experimental procedure that gave reproducible results and decay curves which were straight on a logarithmic scale (Fig. 5) at all RH levels. The strains were inoculated from stock cultures into 10 ml of meat infusion broth and incubated for 8 hr at 37 C. This culture was diluted 1:500, and a standard droplet (0.03 ml) was inoculated into 20 ml of meat broth which was incubated for 16 hr on a turntable at 37 C. This culture was diluted with meat broth to an extinction of $E = 0.180$ (Unicam). A further dilution of 1:100 was made with meat broth. This suspension containing about 6×10^5 viable particles per cubic meter was sprayed with a direct spray (FK 8) with 5 atm of nitrogen (0.5 ml in 4,000 liters). Collection was on double-layered blood-agar plates with 10% sheep blood in a meat broth base. Important features seem to be the incubation under slight agitation, the use of metabolically inert organisms after 16 hr of growth, omission of centrifugation and washing, and the use of a direct spray. Recoveries were between 40 and 50%. At low

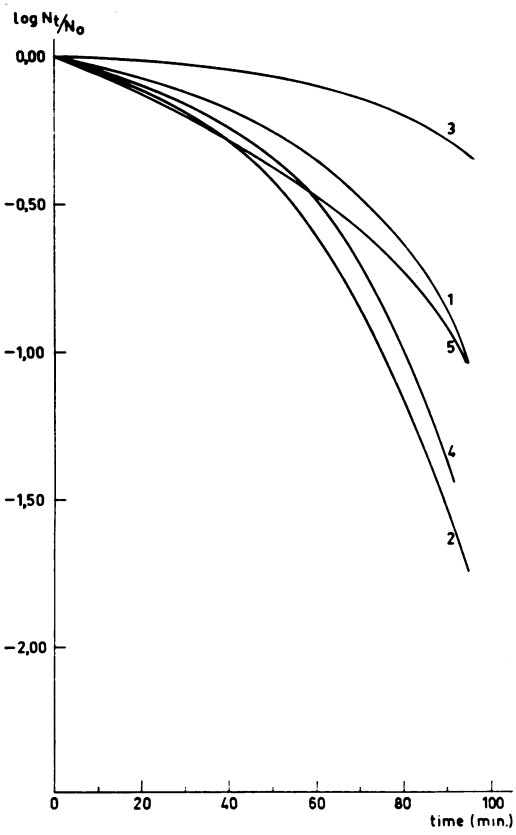

FIG. 4. *Variance of decay curves with culture age (70% RH).*

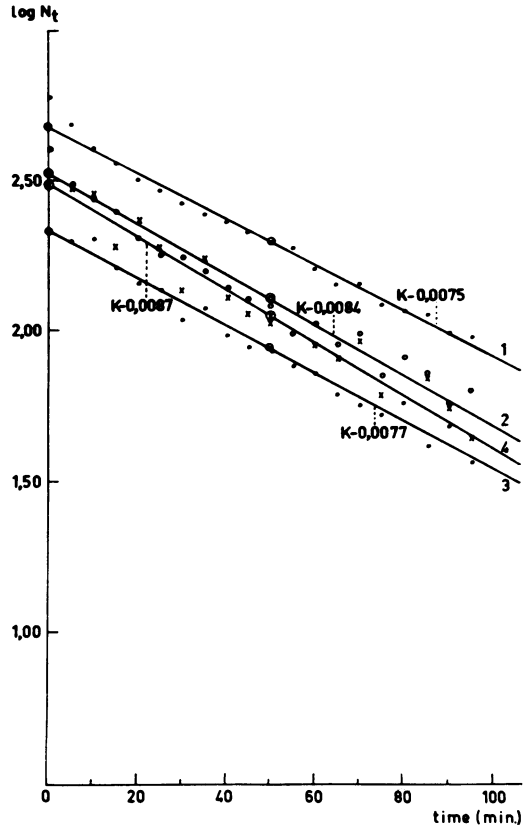

FIG. 5. *Decay curves of staphylococci at 90% RH. Standardized experimental procedure. Curves calculated with least squares. Strains 1, 2, and 3 from Table 1. No. 4 = strain 1600.*

TABLE 2. *Survival in air of strains which had caused epidemic events in hospitals, compared with reference strain 1600 (90% RH)*

Strain	Phage type	$K \times 10^{-4}$	K/K_{1600}
1600	187	85	1.00
3827	80/81	87	0.90
3828	80/81	78	1.03
3829	80/81	77	1.01
3830	80/81	76	1.00
3822	52/52A/80/81	74	0.75
3826	6/7/47/53/54/75/83A/81	76	0.79
3824	6/53/83A	91	0.96
3825	NT type A	84	0.88
3823	83A	91	0.96

RH, they were slightly higher. Differences between strains were negligible. In Table 2, the mean recovery was 48% with $\sigma = 2.4\%$.

The results with a number of strains are given in tables 1 and 2 in terms of total decay rate K ($K = \frac{\Delta \log N}{\Delta t}$, where N = number of organisms and t = time in minutes). Physical fall-out in the system, as tested with spores and with fluorescein, was below $K = 0.002$, but was not subtracted. No obvious difference was observed between strains of various phage types, all isolated from patients (noses, lesions, etc.) or between the strains received by the courtesy of M.T. Parker from the Central Public Health Laboratories at Colindale, London, England, which had given rise to epidemics (Table 2).

The data seem to indicate that "epidemic" strains do not survive better than other strains in air. Considering the difficulties in standardization of experimental conditions and our lack of knowledge of the factors causing the reproducibility, it remains possible that differences between strains are masked by the procedure used.

LITERATURE CITED

1. McDADE, J. J., AND L. B. HALL. 1963. Survival of Staphylococcus aureus in the environment. 1. Exposure on surfaces. Am. J. Hyg. **78**:330–337.
2. MALTMAN, J. R., J. H. ORR, AND N. A. HINTON. 1960. The effect of desiccation on Staphylococcus pyogenes with special reference to implications concerning virulence. Am. J. Hyg. **72**:335–342.
3. MORTIMER, E. A., E. WALIKSKY, A. J. GONZAGA, AND C. H. RAMMELKAMP. 1966. Role of airborne transmission in staphylococcal infections. Brit. med. J. **1**:319.
4. ROUNTREE, P. M. 1963. Infection in hospitals, p. 67. Blackwell, Oxford.