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A Study of Carriers of Staphylococcus Aureus

with Special Regard to Quantitative Bacterial Estimations

By

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Preface

The present study was performed in the years 1961—1964 at The University of Bergen, School of Medicine, Medical Department B and The Gade Institute, Department of Microbiology when I was a Research Fellow at The Medical Department B.

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I. Introduction and plan of study

Before aseptic methods were brought into use, the frequency of infections among patients in hospitals was extremely high. Today, hospital infections are far less common, but in relation to the great progress in medical research in the last decades they still represent a major problem.

A substantial number of hospital infections are caused by yellow, coagulase positive staphylococci — Staphylococcus aureus (here abbreviated to Staph. aureus) (149). These organisms are extremely widespread both inside and outside hospitals. Fifty to seventy per cent of hospital personnel and thirty to fifty per cent of the external population carry these organisms in the vestibule of the nose (87, 117). Staphylococci are also frequently isolated from the throat, faeces, vagina, axillae, perineum and other skin sites (14, 17, 19, 56, 87, 133), as well as from the air, bedclothes, furniture and floors of the wards (56, 87, 99, 125).

Certain staphylococcal strains seem to be more virulent than others and individuals harbouring such strains are assumed to be "dangerous carriers". However, it is reasonable to assume that there is also a quantitative aspect in the development of staphylococcal infections.

Some staphylococcal carriers — the so-called "heavy dispersers" — are able to shed far larger numbers of these organisms than other carriers (55, 99). The mode of transmission of staphylococci is well known (57), but it is not clear why carriers differ in their ability to disperse the organisms into the air (149).

The aim of the present study has primarily been to investigate this quantitative aspect; to study the question of why some staphylococcal carriers disperse much larger numbers of organisms into the air than others.

Measures for the prevention of staphylococcal dispersal in hospitals have frequently been investigated (40, 42, 43, 63, 101, 110, 117). Both personnel and patients have been treated with antibiotics and skin disinfectants. The reduction of bacterial air contamination has been attempted by means of ultraviolet irradiation, glycol vapour, improved ventilation, oiling of floors and bedclothes etc.

The majority of investigations have been undertaken on groups of individuals, e.g. patients in one or more hospital wards. The reduction of bacterial air contamination, frequency of staphylococcal nasal carriers and septic lesions have been indicators of the effectiveness of the treatment (42, 43, 63, 110, 117).

In only a few investigations has attention been focussed on the individual carrier (98, 132). Little is therefore known about the ability of the various remedies to reduce the dispersal from carriage sites and septic lesions, the only true sources of

hospital staphylococci. In the present study, the effect of antibiotic nasal spray and hexachlorophane skin disinfection have been investigated with a view to reducing staphylococcal dispersal from individual carriers.

To study these problems, it has been necessary to develop suitable methods for quantitative estimations of Staph. aureus on different sites of the body and for assessment of the ability of individuals to disperse the organisms into the air.

II. Methods

A. Methods of quantitative estimation of Staph. aureus on different skin areas.

Price (103, 104) and Lovell (81, 82) divided skin bacteria into two groups, "transient" and "resident" flora.

The first group comprises all bacteria which are conveyed to the skin from the surroundings. These organisms are usually loosely attached to the surface of the skin. They are relatively easily removed by ordinary washing with soap and water, scraping the skin with a knife or rubbing with a moistened swab. They are present in greatest numbers on the uncovered parts of the body and particularly under the nails (1), while far fewer organisms can be isolated from covered skin areas. The group includes both saprophytes and various pathogenic bacteria such as gram-positive cocci and enterobacteria. Transient organisms may become resident; this sometimes occurs when the skin is exposed to the prolonged action of infected material.

The resident organisms multiply on the skin. They lie in the outer layer of the epidermis, in the pilonidal follicles and sebaceous glands, but have not been demonstrated in the sweat glands (81). The number of resident organisms seems to be fairly constant for the same individual from time to time (103). They cannot be completely removed by mechanical and chemical means. Generally, they comprise three groups of saprophytes: micrococci, corynebacteria and Propionobacterium acnes (85).

1. *Previous methods.*

The total number of bacteria on a skin area cannot be measured. By the methods available only part of the skin flora can be examined.

In order to assess the effect of skin disinfectants, Price (103) developed a standardized form of hand washing. The hands were washed with soap and brush in a series of bowls of water, and the bacterial count for each bowl was determined. Nearly all the transient bacteria were washed off in two minutes and the total skin flora was reduced by about a half every six minutes. Price (103) maintained that theoretically it would take about two hours of continuous washing to sterilize the skin. His method is probably the most exact for quantitative estimation of the bacterial flora of the hands, but it is very time consuming. Other investigators (20, 37, 106) have used the same method in principle but with fewer washing steps. In still other investigations (83, 84) a standardized form of hand washing without scrubbing has been employed.

Skin contamination has also been assessed by pressing the culture medium against the skin and counting the resultant number of colonies (49, 58, 79, 118). This method lends itself best to the investigation of the bacterial flora of the hands.

Rubbing the skin with moistened swabs subsequently plated on solid media is a method which has often been employed (30, 39, 54, 55, 87). Alginated swabs have been dissolved, for example, in Ringer

solution from which counts of viable organisms have been made (3).

Abrahmson and Smorodinzeff (1) rubbed the skin with sterile gauze and Hægler (70) used a hemp or silk thread. Colebrook (24) swabbed the fingers with sterile broth, making cultivations in poured agar and on the surface of solid media. Burtenshaw (16) applied a rectangular glass chamber to the skin, added saline, and scraped the skin with a glass slide to make a suspension of organisms from which he made viable counts. In principle the same method has been used later (69, 84, 122). Several authors (13, 83) have counted the number of organisms in surgical gloves after use for a given period.

The accuracy of these methods has seldom been investigated. Price (103) examined the results of rubbing the skin with sterile swabs and scraping with a sterile scalpel. He found that "transient bacteria, whether involuntary contamination or test-bacteria, could be pretty effectively recovered by these means, although the results from a quantitative standpoint were not dependable. In testing the resident flora, however, scraping and rubbing (particularly the former) proved unreliable, even from a qualitative standpoint". In these investigations Price did not, however, describe how the quantitative determinations were performed.

The results probably vary considerably from method to method, the variations for each individual method being smaller.

2. *Personal methods.*

In the present study, two methods of quantitative estimation of Staph. aureus on the skin have been employed. In one method, different skin areas were examined by means of moistened cotton wool swabs. The other method was a standardized form of hand washing.

a. Equipment.

Swabs: The swabs were made of steel wire and cotton wool. The steel wire was 20 cm long. The cotton wool, which was wound tightly round one end of the wire, had a diameter of 0.5 cm, the length varying frcm 2 to 10 cm depending on the size of the skin area to be examined.

Test tubes: 16 cm long test tubes with an internal diameter of 14 mm.

Pipettes: Calibrated pipettes, capacity 0.1, 1 and 10 ml.

Fluids: 0.9 per cent saline was used for all dilutions and for moistening the swabs. Ordinary tap-water was used for washing the hands.

Washing bowls: These were about 15 cm deep and held 4 litres.

Measuring cylinder: A 500 ml cylinder was used to measure the washing water.

Brushes: Ordinary nail brushes were employed.

All equipment was sterile.

b. Description.

Method of investigating different skin areas.

The skin areas were firmly rubbed six times with swabs moistened in 0.9 per cent saline. Every second time the swabs were rotated about 120°. Two swabs were used for the same skin area, one after the other.

After obtaining the sample the swabs were put into a test tube containing 10 ml of 0.9 per cent saline and treated in the following manner: the steel wire was held between the first and second fingers and rotated 20 to 30 times each way, as fast as possible, until the cotton wool was freed from the wire. The cotton wool was then gathered round the steel wire and squeezed against the wall of the tube to express the fluid. These operations were performed 5 times with each swab. The swabs were then transferred to a second test tube with 10 ml of 0.9 per cent saline and treated in the same way.

The contents of the tubes were mixed and several of the following volumes of the original suspension incubated on mannitol salt agar plates: 1/20, 1/40, 1/80, 1/1,000, 1/4,000, 1/40,000. Each plate was incubated with 0.25 ml suspension. For each sample 1/20, 1/40 or 1/1,000 of the original suspension was always incubated, together with one or two of the other volumes, depending on the presumed degree of skin contamination.

Clean pipettes were used for each dilution tube. The incubates were distributed over the medium by shaking the plates horizontally. After 10—20 minutes the fluids had dried into the media and the plates were put in the incubator with the lid downwards. After incubation for 48 hours at 37°C, the Staph. aureus colonies were counted and the number of organisms in the original suspension calculated. If a plate yielded more than 200—300 Staph. aureus colonies, one which was incubated with a smaller volume of the original suspension was counted.

Sample from the upper lip. The sample was obtained from a 4 sq. cm area in the middle of the upper lip just below the nose.

Sample from the fingers. The sample was obtained from under the nails and proximally from an area 3 cm wide and 2 cm long on the palmar surface of the fingers. Four swabs were used for each hand, 2 swabs for the 1st and 2nd fingers and 2 swabs for the other fingers.

Sample from the perineum and extra-perineal area. As far as possible, the precise perineal area examined in women was that between the anal orifice and the vaginal introitus and 3—4 cm to each side of the mid-line, and in men the area between the anal orifice and the posterior fold of the scrotum and 3—4 cm to each side. The extra-perineal area constituted here a 5 cm broad field outside the perineum on both sides.

Sample from the axillary regions. The sample was obtained from a 50 sq. cm area in the central part of each axilla.

Sample from the skin of the abdomen. The sample was obtained from 200 sq. cm of the skin in the umbilical region.

Sample from staphylococcal infected skin lesions. The whole infected area was sampled, but on 3 occasions these areas were so large that samples were only taken from a smaller part.

Sample from the auditory meatus. The swabs were rubbed around the walls of the auditory meatus.

Method of investigating the hands.

This method is the same as that described by Lowbury et al. (84), except for minor modifications.

After obtaining the finger samples, the subjects washed their hands in half a litre of water at 40°C. Both hands were thoroughly moistened up to the wrist and then rubbed firmly 3 times palm to palm, 3 times with the right palm over the left dorsum, 3 times with the left palm over the right dorsum and 3 times with the fingers interlacing. The hands were thoroughly rinsed between each of these manoeuvres. The whole procedure was repeated 5 times in the same water. The subjects were supervised throughout the washing, which lasted about four minutes. The following volumes of the water were incubated: 1/500, 1/2,000, 1/10,000 and occasionally 1/100,000. Each plate was incubated with 0.25 ml suspension.

c. Reproducibility.

In patients who were non-carriers of staphylococci (samples obtained from the

Table 1. *Per cent of test bacteria recovered from upper lip of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		46.3	
14.1		29.3	
12.2	12.4	63.7	49.3
11.9		50.5	
12.1		56.6	
4.6		24.0	
4.4		58.7	
4.8	4.5	42.7	40.6
4.2		21.4	
4.5		56.0	
9.4		57.3	
8.3		60.8	
8.6	9.1	18.1	44.3
9.4		56.8	
9.6		28.2	

nose, throat, upper lip, axillae, skin of abdomen and perineum) the described areas of upper lip, 1st and 2nd fingers of one hand, skin of abdomen, axillae and perineum were infected with 0.04 ml and the hands with 1 ml of a suspension of Staph. aureus in physiological saline. For each skin area 3 groups of 5 subjects were infected, and for each group the numbers of infecting organisms were calculated in five samples of the suspension used.

After the skin had dried for 5 minutes, samples were obtained by the methods described. The results are given in tables 1—6. Within each group the variations in the numbers of organisms used for infection were small, but the numbers of organisms isolated from the different subjects varied to a much greater extent. However, compared with the results of the later investigations (chapters IV, V, VI and VII), these variations were of minor importance.

In the later examination of staphylococcal carriers the distribution of the counts from the skin, air and nasal samples was skewed, as commonly occurs in such data (51, 86, 147). The frequency distribution of the counts was found to approximate the log.-normal form. For statistical analysis, the counts were therefore transformed to a logarithmic scale and all computations made on the transformed figures. This was accordingly also done in testing the reproducibility of the technique.

The reproducibility of the technique was calculated for each skin area in 3 groups of 5 subjects. Tested by an analysis of homogeneity of variance according to M. S. Bartletts formula (119) for approximate χ^2-distribution, the group variances did not prove to be significantly different ($P > 5$ per cent). The mean values for each group were then tested by an ordinary one-way analysis of variance. The calculated F-values with $f_1 = 2$ and $f_2 = 12$ degrees of freedom were 0.7121 for upper lip, 0.2238 for fingers, 0.2612 for axillae, 1.8126 for skin of abdomen, 0.2727 for perineum and 0.1195 for hands. The corresponding P-values were 20 per cent for the skin of abdomen and more than 50 per cent for the other skin areas. Consequently, there was no significant difference between the groups. Thus, the reproducibility of the methods was considered to be satisfactory.

Table 2. *Per cent of test bacteria recovered from fingers of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		19.7	
14.1		39.5	
12.2	12.4	34.1	39.0
11.9		56.3	
12.1		45.2	
4.6		66.7	
4.4		32.7	
4.8	4.5	40.0	40.5
4.2		37.4	
4.5		25.8	
9.4		50.7	
8.3		60.8	
8.6	9.1	18.1	40.3
9.4		23.4	
9.6		48.5	

Table 3. *Per cent of test bacteria recovered from axillae of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
9.4		28.7	
8.3		11.5	
8.6	9.1	9.7	19.0
9.4		28.7	
9.6		16.3	
16.7		30.3	
15.9		40.0	
15.9	16.9	9.2	21.9
17.1		19.4	
19.0		10.8	
1.6		52.2	
1.7		34.1	
2.0	1.8	11.4	26.4
1.5		9.1	
2.0		25.0	

The number of staphylococci lost during treatment of the swabs was investigated in the following manner. Five swabs were infected with 0.1 ml of a suspension of staphylococci and treated in physiological saline as previously described. Three such experiments were performed and the number of viable organisms was calculated for each experiment in five 0.1 ml samples of the suspension used. The results are given in table 7. From 50 to almost 100 per cent of the infecting organisms were recovered from the swabs.

From patients who were staphylococcal skin carriers, three consecutive samples were obtained from the same skin site, using swabs in the manner previously described. The results are given in table 8. The first sample always yielded the ma-

Table 4. *Per cent of test bacteria recovered from skin of abdomen of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
11.9		66.6	
14.1		59.2	
12.2	12.4	44.4	57.9
11.9		65.6	
12.1		53.7	
4.6		52.5	
4.4		40.9	
4.8	4.5	49.8	54.4
4.2		56.9	
4.5		72.1	
11.7		36.4	
13.2		73.6	
13.4	13.0	42.0	57.5
12.0		71.4	
14.9		64.1	

Table 5. *Per cent of test bacteria recovered from perineum of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
145.0		58.5	
131.0		30.9	
150.0	145.4	44.7	42.6
144.0		50.2	
157.0		28.9	
15.4		30.1	
15.5		22.3	
15.0	15.9	57.7	42.8
16.3		48.4	
14.6		55.5	
12.9		25.3	
14.5		54.9	
13.7	13.9	74.7	48.7
15.4		30.5	
13.1		56.9	

jority of the staphylococci, only small numbers being isolated in the last sample.

To investigate whether the results obtained by the hand washing technique were representative of the actual number of staphylococci on the hands, 10 patients who were staphylococcal skin carriers performed the following experiment. After the standardized hand washing, they scrubbed their hands for two minutes in a second bowl of water, rinsed them thoroughly in this water and scrubbed their hands again for two minutes in a third bowl. Counts of the viable staphylococci in each bowl were made. The results are given in table 9. The ratio between the numbers of staphylococci in the first bowl, and in the three bowls together, varied between 19 and 66 per cent. There was a significant decrease in the number of organisms from the 2nd to the 3rd hand washing.

d. Discussion.

In consecutive samples obtained from the same skin site, using moistened swabs, the majority of Staph. aureus were demonstrated in the first sample and relatively small quantities or nothing at all in the last test. Compared with the results obtained in experiments using test organisms, these investigations show that the numbers of staphylococci demonstrated by the method described, were representative of the quantities of these organisms in the transient flora where they usually appear (81, 82, 103, 104).

Table 6. *Per cent of test bacteria recovered from hands of 15 individuals.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of of 5 individuals	Mean
188		23.4	
150		24.7	
160	162	49.4	42.0
168		55.6	
146		56.8	
608		43.4	
618		26.2	
606	649	21.0	33.0
692		30.2	
720		44.1	
646		37.8	
722		41.1	
654	661	33.6	32.2
628		46.9	
656		16.0	

Table 7. *Per cent of test bacteria recovered from 15 swabs.*

No. of infecting organisms (in thousands)		Per cent of organisms recovered	
Replicate counts from 3 suspensions	Mean	3 groups of 5 swabs	Mean
1.8		60.3	
1.7		69.0	
1.8	1.9	81.9	73.7
2.1		94.8	
1.8		62.5	
1.3		99.5	
1.8		89.0	
1.3	1.5	89.0	79.6
1.6		49.7	
1.6		70.7	
10.6		81.5	
9.7		72.5	
9.5	9.8	78.6	81.2
9.0		90.9	
10.4		82.3	

Table 9. *Number of staphylococci in 3 consecutive samples from the hands of 10 skin carriers. No. of colonies × dil. (in thousands).*

Pat. no.	Sample no. 1	Sample no. 2	Sample no. 3	Total	1st sample as per cent of total
1	182.0	82.0	14.0	278.0	65.5
2	145.0	75.0	3.5	223.5	64.9
3	36.0	48.0	7.0	91.0	39.6
4	25.0	36.0	2.5	63.5	39.4
5	39.0	41.5	3.5	84.0	46.4
6	90.0	100.0	10.0	200.0	45.0
7	305.0	285.0	61.0	651.0	46.9
8	90.0	80.0	9.5	179.5	50.1
9	110.0	370.0	100.0	580.0	19.1
10	840.0	1,360.0	46.0	2,246.0	37.4

Scrubbing the hands for two minutes removes the transient flora (103). In the present investigation (table 9) the transient flora of the hands (as well as some of the resident flora) should consequently have been removed by the three hand washings. Since 19 to 66 per cent of the total number of Staph. aureus in the 3 hand washings was demonstrated in the first bowl, the results obtained by the standardized hand washing should be representative of the number of staphylococci in the transient flora of the hands.

The considerable drop in the number of staphylococci from the 2nd to the 3rd hand washing, supports the theory that most of these organisms belong to the transient skin flora. Accordingly, the results obtained by this method should also be representative of the total number of Staph. aureus on the hands of most of the skin carriers.

Table 8. *Number of staphylococci in 3 consecutive samples from the same skin area. No. of colonies × dil. (in thousands).*

Pat. no.	Skin area	Sample no. 1	Sample no. 2	Sample no. 3	Total (range)	1st sample as per cent of total (mean)
1	Fingers	0.34	0.02	<0.02	0.38—0.36	91.9
2		0.70	0.16	<0.02	0.88—0.86	80.5
3		1.92	<0.02	<0.02	1.96—1.92	99.0
4		0.08	<0.02	<0.02	0.12—0.08	80.0
5		0.04	<0.02	<0.02	0.08—0.04	66.7
6		105.00	20.00	1.00	126.00	83.3
7		0.88	0.18	0.06	1.12	78.6
8		20.00	3.00	0.28	23.28	85.9
9		0.40	0.10	0.02	0.52	76.9
10		85.00	13.00	0.20	100.00	85.0
11		52.00	9.00	1.00	62.00	83.9
12	Upper lip	0.08	0.02	<0.02	0.12—0.08	80.0
13		1.08	0.12	<0.02	1.22—1.20	89.3
14	Axillae	168.00	120.00	11.00	299.00	56.2
15		680.00	284.00	32.00	996.00	68.3
16	Perineum	1.60	0.44	0.16	2.20	72.7
17		244.00	96.00	4.00	344.00	70.9
18		32.00	7.60	2.00	41.60	76.9
19		56.00	2.80	0.80	59.60	94.0
20		2.08	0.44	0.08	2.60	80.0
21		27,480.00	6,780.00	200.00	34,460.00	79.8
22	Dermal lesion	38,700.00	18,300.00	6,360.00	63,360.00	61.1
23		4,960.00	3,980.00	1,750.00	10,690.00	46.4
24		91,00	27.00	4.00	122.00	74.6
25		44,800.00	13,600.00	6,000.00	64,400.00	69.6

e. Summary and conclusions.

1. A method of quantitative estimation of Staph. aureus on different skin areas and a method of determining staphylococcal contamination of the hands are described.

2. By infecting different skin areas with a suspension of Staph. aureus it was demonstrated that the reproducibility of the methods was satisfactory.

3. The results obtained by the methods were representative of the number of Staph. aureus in the transient skin flora of staphylococcal carriers.

B. Method of quantitative estimation of Staph. aureus in the vestibule of the nose.

1. *Previous methods.*

Nasal cultures have usually been obtained from the vestibule with cotton wool swabs and spread on various solid media or put into broth. Some authors (123) claimed that they achieved a higher frequency of positive samples by so-called "deep swabbing", i.e. obtaining samples from the vestibule and backwards to below the middle concha. On this point, however, opinions are divided. Williams et al. (149) maintained that nothing would be gained by swabbing the higher reaches of the nose, and in fact, that mistakenly swabbing beyond the vestibule might produce artificially low carrier rates. The investigations of Moss et al. (98) support this view.

The quantitative assessment of these organisms in the nasal vestibule has generally been based on a rather crude distribution into three groups (scanty, moderate and abundant) of staphylococcal colonies which grew on solid media stroked by the culture stick. Siboni (117) used a more differentiated distribution into 6 groups. Others (55, 56, 107) have counted those plates which contained less than 1,000 colonies, while those yielding more were grouped as ∞.

White et al. (142) obtained nasal cultures by means of cotton wool swabs moistened in broth. The swabs were put into small tubes containing 3 ml broth and shaken for 5 minutes in a Khan shaker. Serial ten-fold dilutions of the broth were then plated on agar media. After incubation, the pigmented colonies were counted and multiplied by the dilution factor.

Presumably the last method gave the best expression of the number of Staph. aureus in the nasal vestibule.

2. *Personal method.*

a. Description.

Nasal samples were obtained from the entire anterior nares, both lateral and medial walls. First a cotton wool swab (the cotton wool covered 2—3 cm of the end of the swab) moistened in 0.9 per cent saline was rubbed with even pressure and constant rotation five times round the inside of each nostril parallel to the skin. Immediately afterwards the anterior nares were examined in the same way, but this time using a dry swab. The swabs were treated in saline as described for skin samples, and the number of staphylococci calculated.

b. Reproducibility.

It was difficult to implant a known quantity of Staph. aureus in the nasal vestibule. The reproducibility of the technique was therefore tested by obtaining 10 consecutive daily nasal cultures from 4 individuals, who had been shown by 6 samples taken at 3-day intervals to be persistent

Table 10. *Number of staphylococci in daily nasal samples from 4 persistent nasal carriers. No. of colonies × dil. (in thousands).*

Day no.	Individ. no. 1	Individ. no. 2	Individ. no. 3	Individ. no. 4	Mean
1	168	112	212	3,920	1,103
2	856	124	172	920	518
3	160	40	80	6,120	1,600
4	252	104	408	1,960	681
5	160	284	364	4,320	1,282
6	296	324	524	880	506
7	100	76	196	2,240	653
8	1,040	136	280	760	554
9	440	48	764	6,040	1,823
10	960	40	728	4,600	1,582
Mean ...	443	129	373	3,176	(1,030)

nasal carriers (the method of selection was the same as for nasal carriers in chapter IV). The results are given in table 10. The reproducibility of the technique was tested by an analysis of homogeneity of variance according to M. S. Bartlett's formula (119) for approximate χ^2-distribution. The calculated value for χ^2 was 0.433 with three degrees of freedom, and the corresponding P-value was 93 per cent. Consequently, the reproducibility of the technique was considered to be satisfactory.

Five consecutive (immediately after each other) nasal cultures were obtained from 10 patients who were persistent nasal carriers on 6 examinations at 3-day intervals. The numbers of staphylococci in the nasal samples are given in table 11 and the logarithms of the cumulative values in fig. 1. The first two samples yielded considerably larger numbers of organisms than the last two, and the numbers for the 10 nasal carriers decreased approximately in parallel from the first to the last sample.

Table 11. *Number of staphylococci in 5 consecutive nasal samples from 10 persistent nasal carriers. No. of colonies × dil. (in thousands).*

Pat. no.	Sample no.				
	1	2	3	4	5
1 ...	640	960	480	480	120
2 ...	120	40	48	8	4
3 ...	2,880	2,000	840	520	440
4 ...	2,480	1,760	1,320	480	360
5 ...	1,040	680	160	200	120
6 ...	1,040	1,040	480	360	280
7 ...	12,080	7,920	4,200	320	200
8 ...	1,560	1,920	280	160	120
9 ...	5,680	4,440	560	600	760
10 ...	524	244	124	84	40
Mean	2,804	2,100	849	321	244

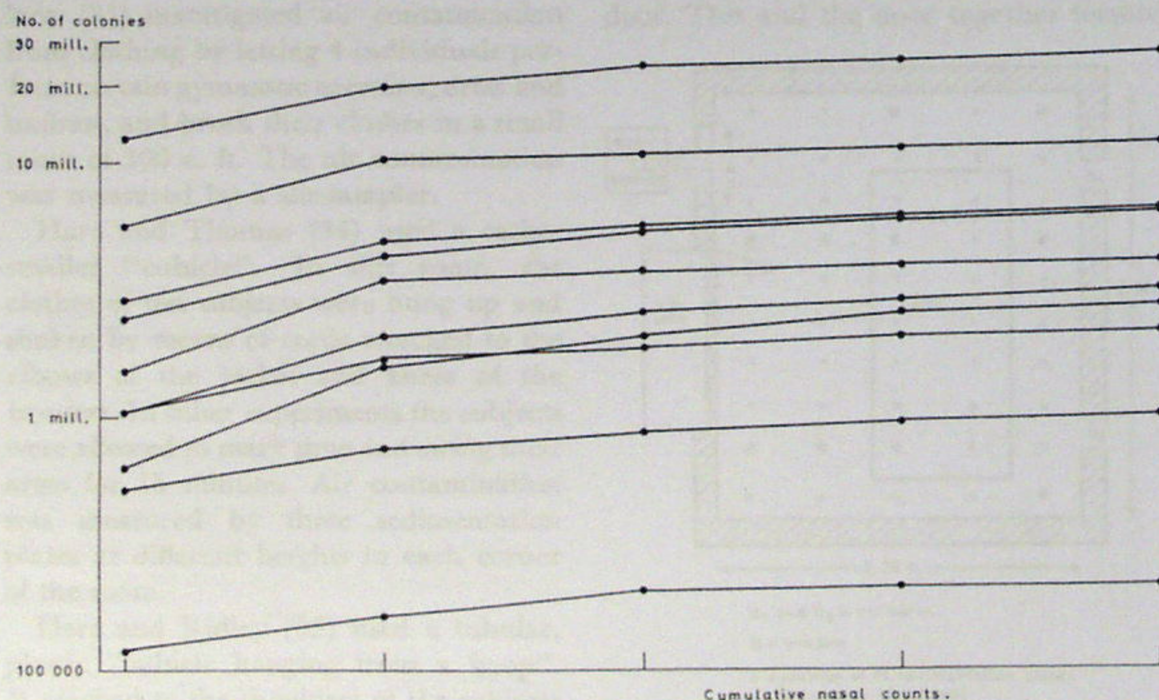

Fig. 1. Cumulative staphylococcal counts in 5 consecutive nasal cultures from 10 nasal carriers.

c. Discussion.

The variations demonstrated in the nasal counts from the 4 individuals (table 10) comprised both variation in the actual number of nasal organisms from day to day and variation in the technique. If a constant number of staphylococci in the nose had been maintained from day to day the changes demonstrated would have expressed only variation in the technique. However, the probability that some of the variations were due to changes in the number of organisms in the nasal vestibule, would indicate that the technique was better than demonstrated in this investigation.

Obtaining several consecutive nasal cultures from the same individual should have eliminated the natural variations in the quantity of nasal staphylococci. If the results given by the method described were representative of the number of staphylococci in the nose, the quantitative variations for nasal carriers from the first to the last sample should be approximately parallel. This was demonstrated by means of 5 consecutive nasal cultures from 10 nasal carriers.

d. Summary and conclusion.

1. A method of evaluating the number of Staph. aureus in the nasal vestibule is described.

2. Experiments with persistent nasal carriers of staphylococci showed that, within wide limits, the results obtained by this method were representative of the number of these organisms in the nasal vestibule.

C. Method of quantitative evaluation of Staph. aureus in the throat.

Throat samples were obtained by dry cotton wool swabs which were rubbed twice over both tonsils and the soft palate and treated in physiological saline as previously described. The number of staphylococci was calculated as described for skin samples.

D. Method of quantitative evaluation of Staph. aureus in the vagina.

Vaginal samples were obtained by dry cotton wool swabs which were rubbed once over the mucous membrane of the vagina. After treatment in physiological saline, 1/1,000 and 1/40,000 of the suspension were incubated on mannitol salt agar and the number of staphylococci calculated.

E. Method of quantitative evaluation of Staph. aureus in the faeces.

1 g of faeces (taken just after defaecation) was collected with a dry swab. The faeces were stirred into physiological saline, 1/1,000 and 1/40,000 of the suspension incubated on mannitol salt agar and the number of staphylococci calculated.

F. Assessment of the ability of individuals to disperse Staph. aureus into the air.

1. *Previous methods.*

It is generally accepted that staphylococcal carriers disperse their organisms into the air mainly on desquamated skin scales (29) from their skin and clothes (31, 54). Direct investigation of the staphylococcal contamination of the patients' clothes would therefore give an indication of their ability to contaminate the air with these organisms. However, a more accurate picture would be obtained by direct measurement of the air contamination caused by bed making, dressing and un-

dressing etc. — activities which are known to cause a major part of the bacterial air contamination in hospitals (10, 57, 73, 87, 99).

Several methods have been employed for quantitative estimation of the bacterial contamination of textiles. Loosli et al. (80) used a slit-sampler as a vacuum-cleaning device and measured the number of organisms removed. Williams' "sweep-plate" method (12) has frequently been used. In the percussion method (96), a weight is dropped onto a piece of cloth stretched over a culture plate. The "contact plate" method (112), rinsing (50), and maceration (115) of a piece of cloth in a suitable fluid are also well known methods.

Air contamination caused directly by staphylococcal carriers in the performance of everyday activities e.g. dressing, undressing, walking, standing still, talking, coughing etc., has been measured only in a few investigations. Duguid and Wallace (31) investigated air contamination from clothing by letting 4 individuals perform certain gymnastic exercises, dress and undress, and brush their clothes in a small room of 100 c. ft. The air contamination was measured by a slit-sampler.

Hare and Thomas (54) used a rather smaller "cubicle". In this room, the clothes of test subjects were hung up and shaken by means of cords attached to the elbows of the jacket and knees of the trousers. In other experiments the subjects were allowed to mark time and swing their arms for 15 minutes. Air contamination was measured by three sedimentation plates at different heights in each corner of the room.

Hare and Ridley (55) used a tubular, plastic "cubicle hanging from a hoop". It reached to the shoulders of the subjects so that the head protruded through an opening in the "roof". Air contamination was measured by 4 sedimentation plates. Dressed in their everyday clothes, the individuals performed the same gymnastic exercises as in the experiments of Hare and Thomas (54).

White (144) investigated the air contamination in the neighbourhood of the patients' beds, using slit-samplers. He tried to observe the conditions generally present in the ward and no attempts were made to increase or diminish activity during the investigation period.

2. *Personal method.*

In the present study, the staphylococcal air contamination caused by a standardized form of bed making in a test room has been investigated.

a. Description.

Fig. 2 shows a drawing of the test room. A plastic wall was put up in front of the door. This and the door together formed

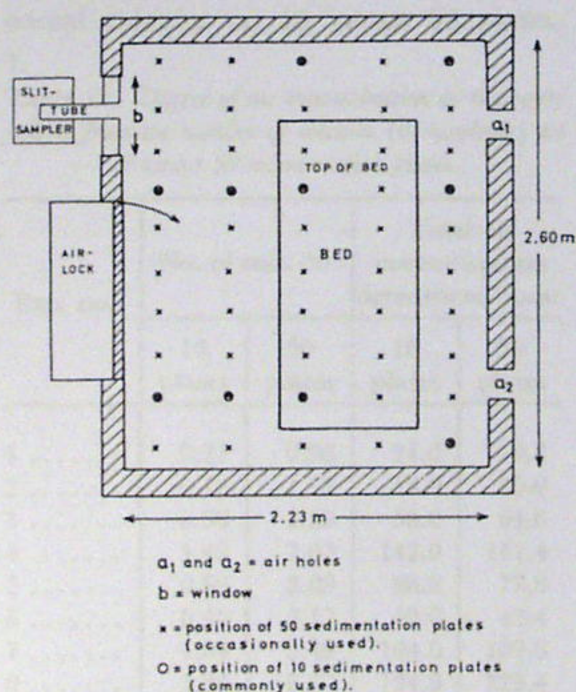

Fig. 2. Test room topography.

a small room (the air-lock) 1.90 m high, 1.05 m long and 0.28 m wide. The plastic wall could be opened by means of a zip. A slit-sampler was connected with the test room by a 40 cm long tube through the wall. The floor was covered with linoleum and the walls and ceiling were lacquered.

After examining 120 c. ft. of the air of the test room with the slit-sampler to ensure that it was not contaminated by staphylococci, the bed was moved into the room and placed as shown in fig. 2. The air-holes and the air-lock were closed and 10 sedimentation plates were disposed on the floor and the bed as shown in fig. 2. The slit-sampler was set in action and, at the same time, a nurse began to make the bed. The eiderdown was folded up and placed over the foot of the bed. The pillows were laid on top of the eiderdown. The sheets were brushed with the hand from the middle of the bed to both sides. The draw-sheet, which lay across the bed, was loosened on one side and shaken twice. Both sheets were secured under the mattress. The pillow and the eiderdown were shaken 3 times, half a metre above the top of the bed, and put back on the bed.

All the beds were made by the same nurse. On repeated examinations of her skin and clothes before she went into the test room, no staphylococci were isolated. The complete bed making lasted $3^1/_2$ minutes. The slit-sampler collected 24 c. ft. of air per minute for 5 minutes. When it was finished, the nurse let herself out through the air-lock. After the bed had been in the test room for an hour, the air-lock and the air-holes were opened, the sedimentation plates were closed and the bed removed.

After incubation of the plates for 48 hours at 37°C the staphylococcal colonies were counted. The 10 sedimentation plates covered 1/100 of the floor of the test room (the surface area of 10 plates was 0.058 sq.m, the floor area being 5.8 sq.m). The total air contamination was calculated by multiplying the number of staphylococcal colonies on the sedimentation plates by 100.

In preliminary experiments (see table 15), it was shown that the number of staphylococcal colonies on the slit-sampler plate was, on average, four times as great as the number on the 10 sedimentation plates. If no staphylococcal colonies were demonstrated on the sedimentation plates, the air contamination was calculated by multiplying the number of colonies on the slit-sampler plate by 25. When there was no growth of Staph. aureus on any of the plates the number of colonies was reported as less than 25 in the tables. However, in statistical calculations, two alternative values were used, namely 24 and 1, the maximum and the minimum number less than 25 (1 is used instead of 0 as the calculations were based on the logarithms of the numbers. $\text{Log.}_{10}\ 1 = 0$, $\log._{10}\ 0 = \div\infty$).

Before the next examination the test room was aired for half an hour and the floor washed twice with a quarter of an hour's interval. The walls and the ceiling were washed every third day with ordinary soap and water.

b. Reproducibility.

Exposure time and number of sedimentation plates. The sedimentation plates were exposed to air contamination for one hour. The question of whether this was adequate time for the greater part of the bacteria-carrying particles to sediment, was investigated in the following experiment. The bed of a heavy staphylococcal disperser was made as previously described. Continuous samples were obtained with the slit-sampler (1 c.ft. per minute) for 36 minutes. Twenty-five minutes later, a

second sample was obtained for five minutes. One hour after the bed making had commenced, the sedimentation plates were removed and new ones put out for 10 hours. Four such experiments were performed. As parallel results were obtained, the results of only one experiment are given in fig. 3. Staphylococcal air contamination

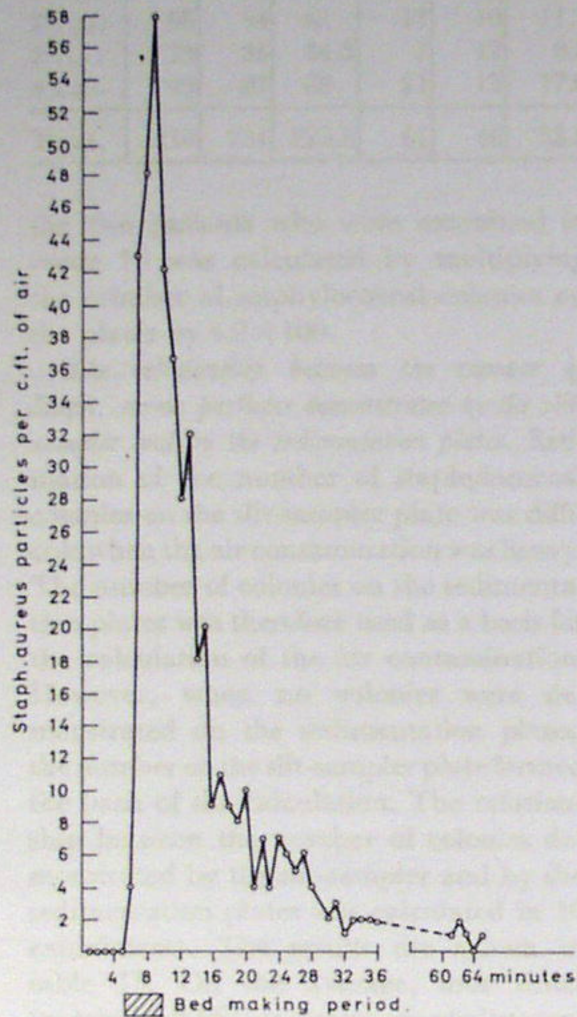

Fig. 3. Air contamination in test room caused by making the bed of a heavy staphylococcal disperser.

was far greater during and just after bed making than 1 hour later. On 10 sedimentation plates exposed for one hour from the commencement of bed making, 1,328 staphylococcal colonies were counted, while only 23 colonies were counted on the 10 new plates put out for the next ten hours. Similar results have been obtained in earlier investigations in the same room (18). Thus, one hour's exposure of the sedimentation plates was sufficient to demonstrate the greater part of the air contamination due to bed making.

To ensure that 10 sedimentation plates were sufficient to give an approximately correct picture of the air contamination, the following experiment was undertaken. A few beds were made as previously described. The staphylococcal air contamination was measured by 50 sedimentation plates evenly distributed over the bed and floor as shown in fig. 2. The plates on the bed were disposed on stands in order not to impede the bed making. Ten of the 50 distributed plates were placed at the same sites as those in the later investigations. Eight such experiments were performed. Table 12 shows the number of staphylococcal colonies on 10 versus 50 plates,

Table 12. *Degree of air contamination in test room judged from the number of colonies (in hundreds) on 10 versus 50 sedimentation plates.*

Exp. no.	No. of cols. on:		Total air contamination determined from:	
	10 plates	50 plates	10 plates	50 plates
1	0.21	0.96	21.0	19.2
2	0.18	1.25	18.0	25.0
3	0.58	3.23	58.0	64.6
4	1.42	7.07	142.0	141.4
5	0.86	3.89	86.0	77.8
6	0.40	2.17	40.0	43.4
7	1.04	5.49	104.0	109.8
8	1.34	6.27	134.0	125.4
Mean ...	0.75	3.79	75.4	75.8

together with the total air contamination calculated on the basis of 10 and 50 plates. The difference in the air contamination calculated from 10 and 50 plates was small and did not justify the use of more than 10 plates.

The reproducibility of the nurse's bed making technique. Air contamination was investigated in a series of preliminary experiments in the test room where six nurses made beds which were artificially contaminated with equal quantities of staphylococcal particles. The mean value for each experiment was calculated and one of the nurses was given the task of practising bed making, with and without patients, so that air contamination was approximately that of the mean values demonstrated. She subsequently made all the beds in the present investigation.

The following test of the reproducibility of her technique was performed. The same areas of the sheets, eiderdown and pillows in six clean beds were contaminated with equal quantities of staphylococcal particles one hour before the beds were made. There was a patient in only one bed (No. 2). Five such experiments were undertaken. The quantity of organisms and the size of the areas of sheets, eiderdowns and pillows which were contaminated, varied from experiment to experiment. The air contamination was measured by 10 sedimentation plates. The results are given in table 13. The variations in air contamination within each experiment were small. The reproducibility of the technique was tested by an analysis of homogeneity of variance based on the logarithms of the observations. It was assumed that the technique was reproducible if there were no difference on the 5 per cent level of significance for each experiment, tested according to M. S. Bartlett's formula for approximate χ^2-distribution. The

Table 13. *Degree of air contamination in test room caused by making artificially contaminated beds. No. of bacterial particles (in thousands).*

Bed no.	Exp. no.					Mean
	1	2	3	4	5	
1	7.9	1.4	2.2	1.9	6.6	4.0
2	4.9	0.7	1.8	1.7	8.4	3.5
3	5.3	0.9	1.9	1.5	6.1	3.1
4	4.7	0.9	2.3	1.1	6.8	3.2
5	7.7	0.6	2.7	1.5	5.4	3.6
6	11.9	1.2	1.1	1.2	4.3	3.9
Mean ...	7.1	1.0	2.0	1.5	6.3	(3.6)

calculated value for χ^2 was 2.5385 with four degrees of freedom and the corresponding P-value was 80 per cent. Consequently, the reproducibility of the technique was considered to be satisfactory.

The relationship between the number of Staph. aureus particles demonstrated on 10 sedimentation plates in the test room and a large room in the department. Two patients had to be examined in a large room (No. 19) in the department because the number of staphylococcal colonies on the sedimentation plates put out in the test room was too large to be counted with accuracy. Room 19 was 4.5 × 4.5 m. In this room, the plates could be placed further from the beds than in the test room.

The relationship between the degree of air contamination demonstrated in the two rooms was investigated by contaminating four beds with equal quantities of staphylococcal particles. Two beds were made in the test room and two in room 19. The results of four such experiments are shown in table 14. Calculated according to the mean values for all experiments, the ratio between the number of colonies demonstrated in the test room and in room 19 was 4.2 : 1. The air contamination by

Table 14. *Degree of air contamination caused by making artificially contamined beds in the test room versus a patient room.*
No. of colonies on 10 sedimentation plates:

Exp. no.	Test room			Patient room		
	Bed		Mean	Bed		Mean
	1	2		1	2	
1	66	54	60	20	11	15.5
2	68	54	61	13	10	11.5
3	33	36	34.5	7	12	9.5
4	49	87	68	21	13	17.0
Total.	216	231	223.5	61	46	53.5

the two patients who were examined in room 19 was calculated by multiplying the number of staphylococcal colonies on the plates by 4.2 × 100.

The relationship between the number of Staph. aureus particles demonstrated by the slit-sampler and by the sedimentation plates. Estimation of the number of staphylococcal colonies on the slit-sampler plate was difficult when the air contamination was heavy. The number of colonies on the sedimentation plates was therefore used as a basis for the calculation of the air contamination. However, when no colonies were demonstrated on the sedimentation plates, the number on the slit-sampler plate formed the basis of the calculation. The relationship between the number of colonies demonstrated by the slit-sampler and by the sedimentation plates was calculated in 10 experiments. The results are shown in table 15. On the average, four times (precisely 3.95) as many staphylococcal colonies were demonstrated by the slit-sampler (24 c.ft. per minute for 5 minutes) as by the 10 sedimentation plates.

c. Discussion.

The air contamination produced by making equally contaminated beds varied little. This is in agreement with investigations undertaken by the percussion method (114). In principle, the technique used in the present study was similar to the percussion method. Hare and Ridley (55) found it difficult to make the test subjects use a uniform degree of activity while exercising in the cubicle. In the present study this difficulty was avoided by letting the same nurse make all the beds.

The number of organisms demonstrated in air samples decreases with the square of the distance from the bacterial spreading process. The plates and beds were therefore placed in fixed positions, so that the distance should be constant in all experiments.

d. Summary and conclusion.

1. To assess the ability of individuals to disperse staphylococci into the air, a standardized form of bed making in a test room is described. The air contamination was measured by sedimentation plates and a slit-sampler.

Table 15. *Degree of air contamination in test room. Slit-sampler (24 c. ft./min. for 5 min.) versus 10 sedimentation plates (exposed for 1 hour).*
No. of colonies.

Exp. no.	Slit-sampler A	Sed.plates B	Ratio $\frac{A}{B}$
1	19	5	3.8
2	60	13	4.6
3	49	12	4.1
4	87	24	3.6
5	66	20	3.3
6	9	2	4.5
7	11	3	3.7
8	13	3	4.3
9	23	4	5.8
10	26	6	4.3
Mean ...	36.3	9.2	4.0

2. By making artificially contaminated beds it was demonstrated that the reproducibility of the technique was good.

G. Bacteriological technique.

The samples were plated on mannitol salt agar medium. After incubation for 48 hours at 37°C, the staphylococcal colonies were counted. Five to ten colonies from the air samples, and 1—3, generally 2, colonies from the other samples, were plated on blood agar. After incubation of the blood agar plates for 18 hours at 37°C the subcultures were examined for coagulase production. All coagulase positive colonies were transferred to broth and antibiogram determinations and phage typing were carried out. If antibiograms and phage patterns left any doubt as to whether two strains from the same individual were identical, serological typing was performed.

1. *Culture media.*

The mannitol salt agar medium was described by Chapman (22). This medium inhibits the growth of gram-negative rods and B. subtilis (21, 22, 38) and the staphylococcal colonies are well coloured (21).

The degree of accuracy by which the Staph. aureus colonies were distinguished from other bacterial colonies on this medium was investigated in the following manner. The staphylococcal colonies and other bacterial colonies were counted on 10 mannitol salt agar plates which contained samples of skin and air contamination from 5 patients. Fifty-seven definite and 3 uncertain Staph. aureus colonies were counted, and 334 colonies of other bacteria. All colonies were submitted to further examinations (acid and coagulase production, microscopic examinations etc.). The first group of 57 colonies were all coagulase positive staphylococci and the other 337 colonies were found to be other bacteria.

In the later experiments, the same classification of the colonies was undertaken. All uncertain colonies were examined for coagulase production. In consequence, the number of wrongly diagnosed colonies should be small and of minor importance in the results of the present investigation.

The blood agar medium was composed of:

1.8 per cent agar, Kobe.
1.0 per cent peptone, Witte.
0.5 per cent sodium chloride (May and Baker).
7.0 per cent human blood.

Meat extract (0.5 kg beef in 1,000 ml water) ad 1,000 ml. ph adjusted to 7.5.

This was used as a subculture medium to avoid mannitol fermentation products which might impede the coagulase test (15, 131).

2. *The coagulase test.*

To rabbit serum stabilized with sodium citrate and diluted 1 : 5 with broth, a loop of blood agar culture was added, shaken and incubated at 37°C. Each tube contained 1 ml fluid. After 3, 4 and 24 hours the results were recorded. Negative tests were examined twice. The capacity to coagulate plasma has been used as the only criterion for the selection of pathogenic staphylococci.

3. *Antibiogram determinations.*

The disc method (36, 128) was employed to determine the sensitivity to sulphathiazole, penicillin, streptomycin, tetracycline, erythromycin and chloramphenicol, using sensitivity discs manufactured by Klinisk-bakteriologiska Laboratoriet, Karolinska sjukhuset, Stockholm.

Three to five ml of a broth suspension of Staph. aureus suitable for producing discrete colonies was transferred to peptone-free blood agar plates containing 1

Table 16. *Ratio between inhibition zone diameter and sensitivity to antimicrobial agents.*

Degree of sensitivity	Inhibition zone (range; mm)					
	Sulpha-thiazole	Penicil-lin	Erythro-mycin	Strepto-mycin	Chloram-phenicol	Tetra-cycline
Highly sensitive	29–35	31–44	26–46	20–35	22–44	21–38
Fairly sensitive	23–28	21–30	20–25	16–19	16–21	17–20
Relative resistant	17–22	12–20	12–19	12–15	11–15	9–16
Resistant	5–16	5–11	5–11	5–11	5–10	5– 8

per cent glucose. The plates were first decanted and the excess fluid pipetted off and then dried horizontally for 30 minutes at 37°C. The discs were placed aseptically on the substrate at least 4 cm apart. The plates were incubated for 18—20 hours at 37°C and the diameter of the inhibition zone was measured. The sensitivity was divided into (1) highly and (2) fairly sensitive groups, and in (3) relatively resistant, and (4) resistant groups. The ratio between the diameter of the inhibition zone in millimetres and the sensitivity to the antimicrobial agents is given in table 16.

Two staphylococcal colonies from the same individual were assumed to be different strains if their antibiograms differed by 2 or more groups.

4. *Phage typing.*

The phages used were the basic set (21 phages) obtained from the Staphylococcus Reference Laboratory, Colindale, except phage 73 which was excluded at a meeting of The International Committee on Phage Typing of Staphylococci in Stockholm in 1959. In addition to these, phages 81 (25), 82 (25), KS6 (139), 83A (136) and 83B (136) were used. The series therefore included 25 phages which were divided into the 5 groups listed in table 17. While most of the staphylococcal strains were lysed by more than one phage, several strains were lysed only by phage 42D or 187. They were placed in two separate groups, IV and V. Strains lysed by phages belonging to different groups have been placed in the "miscellaneous" group.

Table 17. *Group distribution of phages.*

Phage groups	Phages
I	29, 52, 52A, 79, 80, 81, 82, KS6
II	3A, 3B, 3C, 55, 71
III	6, 7, 42E, 47, 53, 54, 75, 77, 83A, 83B
IV	42D
V	187

The notations for recording the degrees of lysis given by the phages are shown in table 18. All lytic reactions from over 50 plaques to confluent lysis were regarded as "strong" reactions.

Table 18. *Phage typing notations.*

+++	"Complete lysis".
++	"Strong lysis". More than 50 plaques.
+	"Moderate lysis". 20—50 plaques.
±	"Weak lysis". Less than 20 plaques.

Strains which were not lysed by phages in the Routine Test Dilution (RTD) were retyped using phages 1,000 times more concentrated (RTD × 1,000). Non-typable strains were recorded as NT (non-typable).

Two cultures were considered to be different when one was lysed strongly by at least two phages which produced no degree of lysis of the other (2, 11, 146). Sometimes (about 1 per cent), subcultures from the same colony differ by more than one "strong" reaction (146). In the present study, strains which were presumed to be identical, e.g. demonstrated in the nose and skin samples from the same individual, occasionally differed by two "strong" reactions, while the antibiograms were identical. In such cases, several colonies (5—15) from the samples were phage typed. If there were still doubt whether the original strains were identical, serological typing was performed.

The phage typing was performed at The Gade Institute, Department of Microbiology.

5. *Serological typing.*

Serological typing was performed according to the technique described by Oeding (100). Factor sera *m* (59), *n* (59), *i* (59), a_5 (60), h_2 (62), c_1 (61), 263—1 (67) and 263—2 (67) were employed. Two strains were considered to be different if they differed in one or more antigens.

The serological typing was performed at The Gade Institute, Department of Microbiology.

III. Experimental design

The present investigation deals with examinations of staphylococcal carriers among the patients admitted to Medical Department B, Haukeland Hospital, Bergen. The subjects were examined once daily, usually for three consecutive days. Further details concerning the selection of patients and number of examinations are given in chapters IV, V, VI and VII.

Two days before the first examination the patients were bathed, or, if bedridden, washed on a stretcher. They were given clean bed and washing equipment, clean clothing and handkerchiefs. Qualitatively and quantitatively all subjects received the same bed equipment (1 mattress, 1 eiderdown, 2 pillows and 2 sheets) and clothing (2 shirts, 2 pairs of trousers and a bathrobe).

To prevent contamination of the patients by staphylococci from their surroundings, they were isolated from other staphylococcal carriers and the bed equipment and clothing were thoroughly examined for bacterial contamination before use.

The subjects were examined between 7.30 and 11.30 A. M. From the time they had gone to bed in the evening (8—9 P. M.) until they were examined the next morning they were not permitted to wash themselves or to leave their beds. When the examination was completed they were permitted to conduct themselves as usual in their rooms.

IV. Self-contamination and dispersal of Staph. aureus by nasal carriers

A. Previous investigations.

The frequency of staphylococcal nasal carriers has been thoroughly examined and most investigations dealing with this problem have been reviewed by Lund (87) and Siboni (117). The carrier rate for adults lies between 30 and 50 per cent, higher nasal carrier rates being demonstrated in hospitals. The number of staphylococci in the nasal vestibule varies considerably from individual to individual, but reports on the quantitative aspects of the problem are few (55, 132, 133, 142, 143, 144).

Only a few staphylococci are expelled directly into the air from the nose and mouth (31, 54, 87, 117). It seems that dispersal of Staph. aureus by nasal carriers depends primarily upon the transfer of organisms from the site of multiplication in the nose to the skin, clothing and bedding by means of fingers and handkerchiefs (54, 57). The pathway can be demonstrated by painting the anterior nares with a fluorescent substance (54).

Accordingly, nasal carriers are frequently also staphylococcal skin carriers in contrast to individuals not carrying the organisms in the nose (39, 54, 87, 89, 97), and the nasal and skin strains are usually identical (55, 87, 93, 97, 132, 144, 145).

Staphylococci have been isolated from the fingers (55, 68) and nail walls (87) of 70 to 90 per cent of nasal carriers; less frequently from the face and back of the wrists (30, 55, 97), and rather seldom and in smaller numbers from the axillae, chest, abdomen, inguinal folds and legs (54, 55, 145).

In dispersal tests on 300 children, Laurell and Wallmark (77) demonstrated that Staph. aureus could be isolated more frequently from the upper lip, hands and clothes of individuals with large quantities of these organisms in nasal cultures than from those with small quantities. Similar results have been reported more recently (132, 144). The number of staphylococci isolated from different skin areas of the same nasal carrier, and from identical skin areas of different nasal carriers, varies considerably. Little is known of the reason for this but reports on the quantitative aspects of the problems are few (55, 57).

Staphylococci are mainly liberated into the air by agitation of clothing and friction of the skin (31, 54). Hare and Ridley (55) reported that nasal carriers who exercised in a cubicle, wearing their ordinary everyday clothing, usually dispersed much larger numbers of staphylococci into the air than non-nasal carriers. However, the ability of nasal carriers to disperse varied greatly, and there was very little correlation between nasal counts and aerial dissemination. In 14 of 16 cases, identical strains were isolated from the nose and air samples. Besides being a nasal carrier, one of the two remaining individuals was

a perineal carrier of a strain different from that isolated from his nose, and the perineal strain was dispersed.

White (143) isolated staphylococci more frequently from the clothing of patients with high nasal counts than from those with low counts. Later he reported that more than 20 staphylococcal colonies per c.ft. of air could be recovered from 35 per cent of air samples obtained after shaking the bed sheets of nasal carriers of large numbers of Staph. aureus, but from only 8 per cent of air samples taken around non-carriers or carriers of smaller numbers (144).

Nasal carriers are frequently throat carriers (76, 133, 134), faecal carriers (14, 17, 66, 91, 92, 138), and sometimes also perineal carriers (19). Dispersal of staphylococci most probably also takes place from these sites. Sufficient attention has not been paid to this factor in earlier investigations of staphylococcal dispersal by nasal carriers.

In the present study, the difference in the ability of nasal carriers to disperse staphylococci was investigated. Possible correlations between the numbers of staphylococci in the nose and on different skin sites and the dispersal into the air were studied.

B. Personal investigations.

1. *Material and methods.*

The material, consisting of 52 women and 48 men between the ages of 14 and 75 years, was selected in the following way. All patients (2,014 in all) admitted to The Medical Department B from August 1962 to October 1963 were examined for staphylococci in the nose and throat and on the perineum. Thirty-six per cent of the patients were nasal carriers at the time of admission. From these patients 4—6 nasal cultures were obtained at two- to three-day intervals. On the last examination, the numbers of staphylococci in the axillae, vagina, perineum and faeces were estimated.

Patients who yielded staphylococci in the nose on all examinations were regarded as predominantly nasal carriers, provided they did not have staphylococcal lesions or too many organisms (1,000 or more Staph. aureus) in samples from the axillae, vagina, perineum or faeces. These criteria will be evident from the investigations reported in chapters VI and VII.

One hundred and eleven patients fulfilled the criteria mentioned. However, 9 patients were unco-operative or too ill to be examined, and in two patients the nasal staphylococci disappeared during the course of the subsequent investigations.

The remaining 100 patients were examined once daily for three consecutive days. In order to keep experimental conditions approximately equal for all the patients, they were bathed, or washed on a stretcher, and received clean clothing and bedclothes two days before the first examination.

The methods of investigation are described in chapter II.

2. *Results.*

a. Nasal samples.

Quantitative estimations.

The numbers of staphylococci in the nasal cultures of the 100 patients are given in the appendix table. The counts varied greatly from individual to individual, the lowest and highest counts being 960 and 32.08 mill. respectively. However, the variations on repeated examinations of the same individual were relatively moderate.

The mean counts for the first, second and third examinations of the 100 patients

Table 19. *Nasal count by age, sex and confinement to bed. 100 nasal carriers.*

Nasal count	Mean age (46.1 years)	No. of patients				
		Sex distribution		Total	In bed	
		F (52)	M (48)		No. (58)	Per cent of total
$<10^4$....	43.0	1	2	3	1	50
10^4-10^5....	45.4	5	8	13	7	
10^5-10^6....	46.3	16	18	34	20	59
10^6-10^7....	46.1	27	16	43	24	56
$>10^7$....	47.1	3	4	7	6	86

varied little and were 2.805 mill., 2.484 mill. and 2.686 mill. staphylococci respectively, the mean count for all 300 examinations being 2.659 mill.

The material was divided into 5 groups on the basis of the mean nasal counts. Group 1 included counts less than 10^4 bacteria, group 2 counts between 10^4 and 10^5, group 3 counts between 10^5 and 10^6, group 4 counts between 10^6 and 10^7 and group 5 counts larger than 10^7 bacteria. Table 19 gives the distribution of sex, average age and number of bedridden patients (out of bed for less than 2 hours daily) for each group. The majority of patients belonged to groups 3 and 4. Group 4 had an excess of women. The average age varied little from group to group, but the frequency of bedridden patients was greatest in the group with the highest nasal counts.

Nasal cultures yielding more than 10^7 staphylococci (group 5) were almost pure cultures, while those with less than 10^5 staphylococci (groups 1 and 2) always consisted of a mixture with other organisms (Staphylococcus albus, Micrococcus catarrhalis and others), these usually constituting the greater part of the organisms. Groups 3 and 4 included the transition cases.

The individuals in group 5, who had the highest nasal counts, were more debilitated by their disease than the remaining patients. Two of the seven patients in this group died during their stay in hospital, one from uraemia and the other from haemolytic anaemia. Only two of the 93 patients in the other groups died while in hospital.

Antibiogram and phage patterns.

On the average, antibiogram determinations and phage typing were performed on 7—8 colonies (ranging from 5 to 23) from the nasal cultures of each patient. In all, 112 strains were demonstrated. Eighty-nine patients had 1 strain, 10 patients had

Table 20. *Drug sensitivity of nasal strains in relation to nasal count.*

Nasal count	No. of strains		Resistant as per cent of total	
	Total	Resistant		
$<10^4$	3	0	0.0	5.9
10^4-10^5	14	1	7.1	
10^5-10^6	37	11	29.7	
10^6-10^7	50	20	40.0	44.8
$>10^7$	8	6	75.0	
Total ...	112	38	33.9	

Table 21. *Phage grouping of nasal strains in relation to nasal count. (No. of strains).*

Nasal count	Typable (phage groups)							Non-typable	Tot.
	I		II	III	IV	V	Miscellaneous		
	80/81	Others							
$<10^4$	0	0	1	0	0	1	1	0	3
10^4-10^5	0	6	0	3	0	0	1	4	14
10^5-10^6	3	10	1	9	0	2	7	5	37
10^6-10^7	4	18	5	12	1	5	3	2	50
$>10^7$	2	1	1	2	0	1	1	0	8
Total	9	35	8	26	1	9	13	11	112

2 strains and 1 patient had 3 different strains.

Seventy-four strains were sensitive to all antimicrobial agents used. Seventeen strains were resistant to penicillin alone. Twenty-one strains were resistant to sulphathiazole, 20 to penicillin, 13 to streptomycin, 9 to tetracycline, 3 to erythromycin and 2 to chloramphenicol. Table 20 gives the results of antibiogram determinations in relation to nasal count. The frequency of strains resistant to one or more antimicrobial agents increased with rising numbers of staphylococci in nasal cultures.

On phage typing, 65 strains were lysed by RTD, 36 strains by RTD × 1,000 and 11 strains were non-typable. Table 21 gives the results of phage typing in relation to staphylococcal nasal count. Patients with the highest nasal counts (groups 4 and 5) yielded less non-typable but more 80/81-strains than those with the lowest counts (groups 1, 2 and 3). The majority of strains belonged to phage groups I and III.

b. Throat samples.

Staphylococci were also isolated from the throat of 51 of the 100 nasal carriers. The numbers of bacteria in throat cultures are given in the appendix table. The counts varied considerably from patient to patient, and also on repeated examinations of the same patient.

Antibiogram determinations and phage typing were performed on 1—2 colonies from each positive sample. In all, 104 colonies were examined and 53 strains demonstrated. Forty-nine patients had 1 strain and 2 patients had 2 strains.

Eighteen strains were resistant to one or more antimicrobial agents. As seen in table 22, the frequency of resistant strains in throat samples was slightly greater for patients with the highest nasal counts (groups 4 and 5) than for those with the lowest counts (groups 1, 2 and 3).

On phage typing, 31 strains were lysed by RTD, 15 by RTD × 1,000 and 7 strains were non-typable. Table 23 gives the phage grouping of staphylococci in throat cultures in relation to nasal count. No definite correlation was observed. The majority of strains belonged to phage group I.

Thirty-five patients had identical strains in the nose and throat while 16 had different strains.

Table 22. *Drug sensitivity of throat strains in relation to nasal count.*

Nasal count	No. of strains		Resistant as per cent of total
	Total	Resistant	
$<10^4$	1	0	28.5 (groups $<10^4$ to 10^5-10^6)
10^4-10^5	4	2	
10^5-10^6	16	4	
10^6-10^7	26	6	34.4 (groups 10^6-10^7 and $>10^7$)
$>10^7$	6	5	
Total ...	53	17	32.1

Table 23. *Phage grouping of throat strains in relation to nasal count. (No. of strains).*

Nasal count	Typable (phage groups)						Non-typable	Total
	I	II	III	IV	V	Miscellaneous		
$<10^4$	0	0	0	0	0	0	1	1
10^4-10^5	2	0	1	0	0	0	1	4
10^5-10^6	7	1	4	0	0	2	2	16
10^6-10^7	12	4	2	1	3	2	2	26
$>10^7$	1	1	2	0	1	0	1	6
Total ...	22	6	9	1	4	4	7	53

c. Faecal samples.

In selecting the material, patients with staphylococci in the faeces were excluded from further investigation. Nevertheless, 6 of the 100 patients (Nos. 9, 34, 42, 79, 84 and 97) yielded from 2,000 to 22,000 staphylococci per g of faeces. Antibiogram determinations and phage typing were performed on 2 colonies from each sample. Four patients had identical strains in the nose and faeces, one patient yielded identical strains in the throat and faeces and one patient had a different strain in the faeces from that in the nose and throat.

d. Skin samples.

Quantitative estimations.

The numbers of staphylococci demonstrated on the upper lip, fingers and hands of the 100 nasal carriers are given in the appendix table. The highest counts for single observations were 41,000 for the upper lip, 457,000 for the fingers and 1.358 mill. for the hands.

Table 24 gives the frequency of positive skin samples in the 3 examinations. In each examination, the frequency was approximately equal for identical skin areas and was highest for the fingers.

Table 24. *Frequency of positive skin samples from 100 nasal carriers.*

Area	Examination no.			At least one positive
	1	2	3	
Upper lip	70	75	74	89
Left fingers............	83	81	87	96
Right fingers	54	56	47	77
Hands	77	76	78	90
Total area	90	91	90	96

Table 25. *Mean numbers of staphylococci isolated from various skin areas of 100 nasal carriers.*

Area	Method of calculation	Examination no. 1	2	3	Mean
Upper lip	I	1,084	1,934	1,304	1,440
	II	1,089	1,939	1,308	1,445
Left fingers	I	6,505	9,199	6,225	7,310
	II	6,508	9,202	6,228	7,313
Right fingers	I	1,363	1,747	1,018	1,376
	II	1,371	1,755	1,027	1,384
Sum fingers	I	7,867	10,946	7,242	8,685
	II	7,879	10,958	7,254	8,697
Hands	I	40,225	36,640	46,715	41,193
	II	40,340	36,760	46,825	41,308

The frequency increased with the number of examinations, but only to a negligible degree with the number of skin areas examined.

Table 25 gives the arithmetic mean counts for each skin area in the first, second and third examinations of the 100 patients, and for the 300 individual observations. For counts below 20 for the fingers and upper lip, and below 500 for the hands, the calculations are based on two alternative values, 1 and 19, and 1 and 499 respectively. In calculation method I, the values are taken as 1, and in method II, as 19 and 499. The difference in the results based on methods I and II was small. The greatest number of staphylococci was demonstrated on the hands and the smallest on the upper lip. The mean counts for identical skin areas varied little on the 3

Table 26. *Correlation between staphylococcal counts from nose and upper lip. Three examinations of 100 nasal carriers.*

Nasal count	No. of examinations	Upper lip: < 20 bact./sample No.	Per cent	≥ 1,000 bact./sample No.	Per cent
$<10^4$....	13	12	92.3	0	0.0
10^4-10^5....	34	30	88.2	0	0.0
10^5-10^6....	117	31	26.5	13	11.1
10^6-10^7....	115	8	7.0	37	32.2
$>10^7$....	21	0	0.0	16	76.2
Total	300	81	27.0	66	22.0

Table 27. *Correlation between staphylococcal counts from nose and fingers. Three examinations of 100 nasal carriers.*

Nasal count	No. of examinations	Fingers: < 20 bact./sample No.	Per cent	≥ 1,000 bact./sample No.	Per cent
$<10^4$....	13	10	77.0	0	0.0
10^4-10^5....	34	14	41.2	1	2.9
10^5-10^6....	117	8	6.8	22	18.8
10^6-10^7....	115	3	2.6	82	71.3
$>10^7$....	21	0	0.0	19	90.5
Total	300	35	11.7	124	41.3

Table 28. *Correlation between staphylococcal counts from nose and hands. Three examinations of 100 nasal carriers.*

Nasal count	No. of examinations	Hands: < 500 bact./sample No.	Per cent	≥ 5,000 bact./sample No.	Per cent
$<10^4$....	13	13	100.0	0	0.0
10^4-10^5....	34	25	73.5	0	0.0
10^5-10^6....	117	28	23.9	33	28.2
10^6-10^7....	115	3	2.6	98	85.2
$>10^7$....	21	0	0.0	21	100.0
Total	300	69	23.0	152	50.7

Table 29. *Correlation between staphylococcal nasal and skin counts. Correlation coefficients based on logarithms of observations. (100 nasal carriers).*

Calculation based on	Method of calculation	N : U	N : F	N : H
3 replicate observations (at one-day intervals)	I	0.6907	0.7500	0.7659
	II	0.6455	0.7431	0.7761
Mean	I	0.7807	0.8502	0.8496
	II	0.7381	0.8218	0.8223

N = nose, U = upper lip, F = fingers and H = hands.

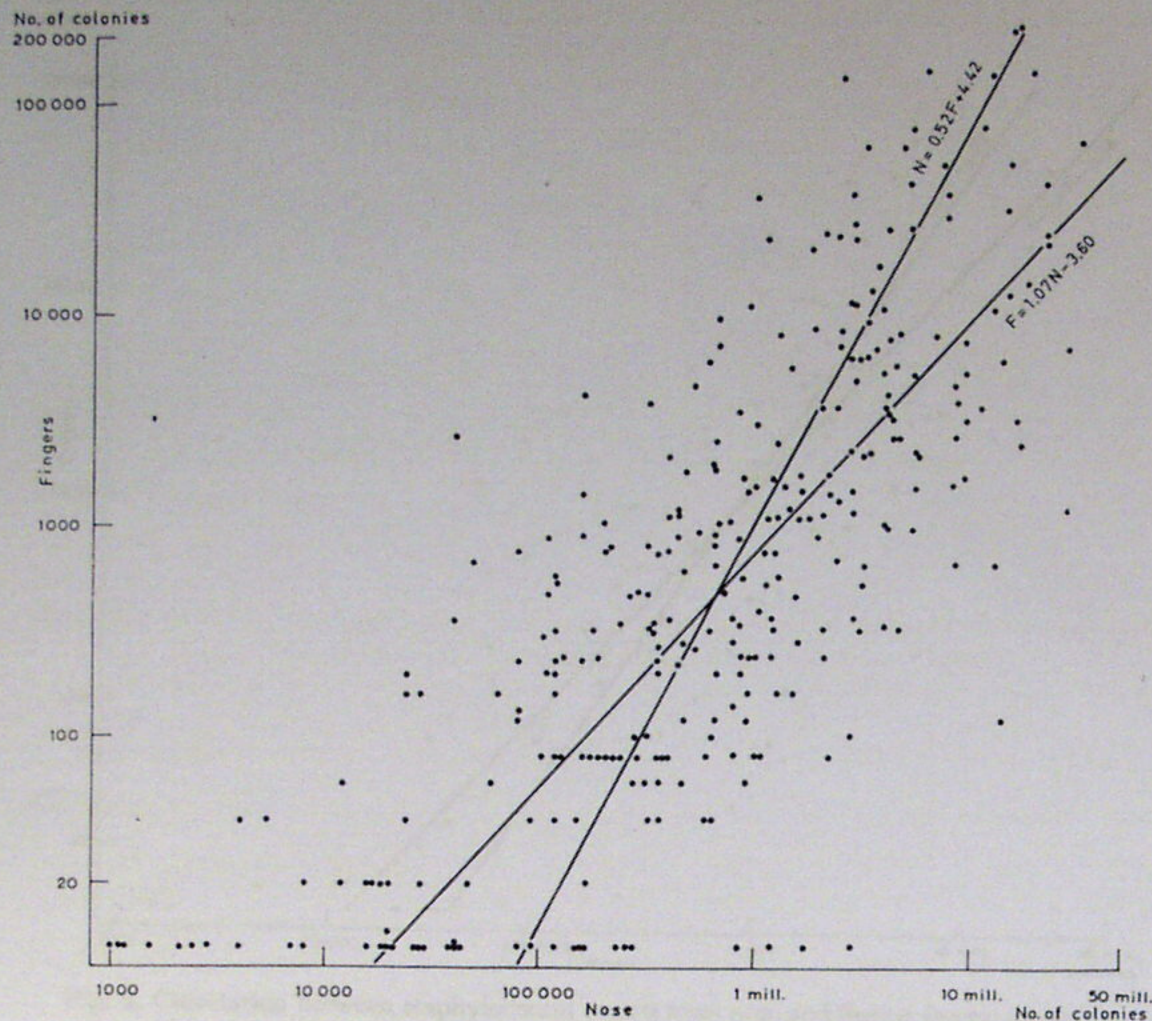

Fig. 4. Correlation between staphylococcal counts from nose and fingers (single counts, 100 nasal carriers).

examinations. More staphylococci were isolated from the fingers of the left hand than from the right.

Tables 26—28 show the correlation between the nasal counts and the counts from upper lip, fingers and hands respectively on 3 examinations of the nasal carriers. Within wide limits, the skin counts increased with rising nasal counts.

The frequency distribution of the skin and nasal counts was found to approximate the log.-normal form. For statistical analysis, the counts were therefore transformed to a logarithmic scale and all computations made on the transformed figures. For counts below 20 for the fingers and upper lip, and below 500 for the hands, the calculations were based on two alternative values as mentioned above.

Table 29 gives the correlation coefficients between logarithms of nasal and skin counts. The correlations were good, being best between the nose and hands and least good between the nose and upper lip.

Fig. 4 illustrates the correlation between logarithms of single counts from nose and

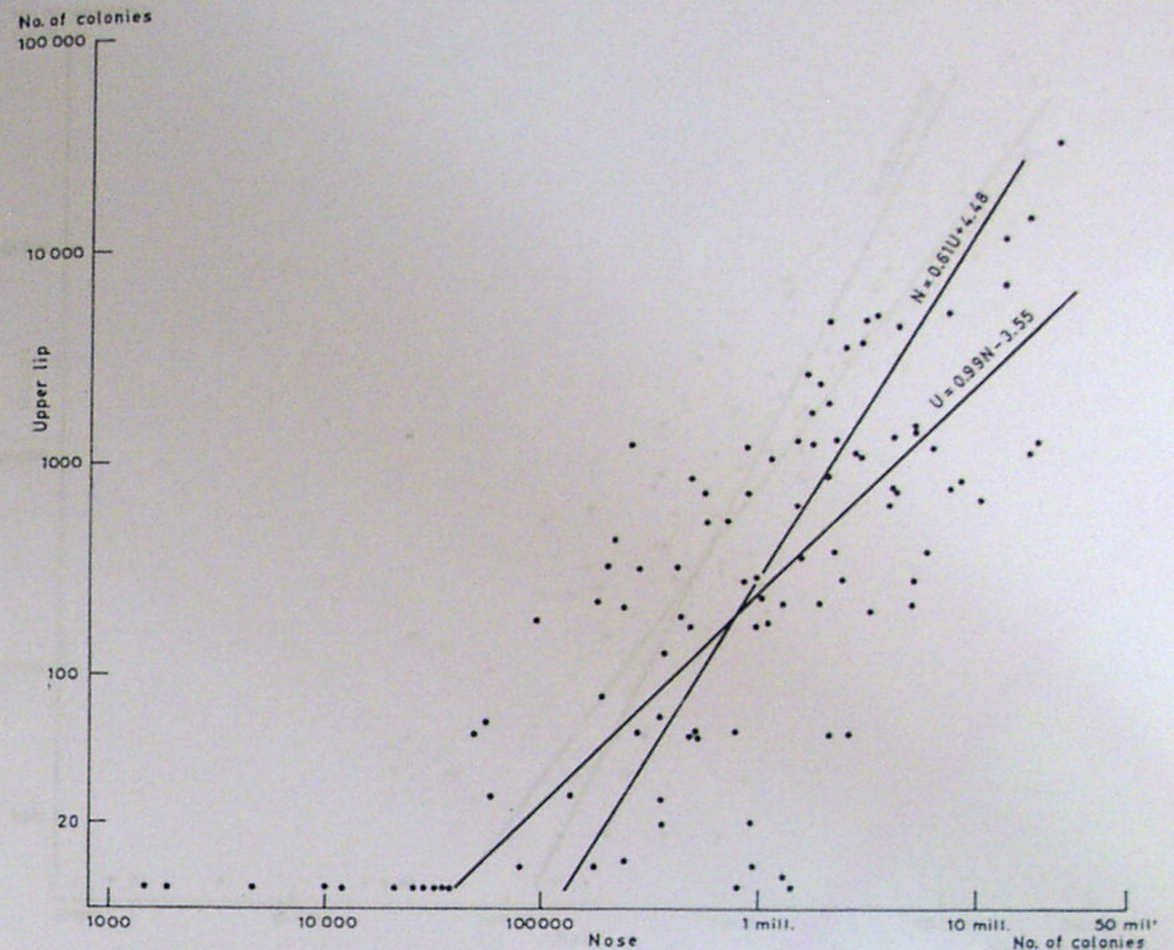

Fig. 5. Correlation between staphylococcal counts from nose and upper lip (mean counts, 100 nasal carriers).

fingers, and figs. 5—7 of mean counts from nose and upper lip, fingers and hands respectively. The mean counts and regression lines marked are calculated on the basis of method I. Values for single counts below 20 and mean counts below 10 are plotted on the abcissa. Within wide limits, the numbers of staphylococci in skin samples increased with rising nasal counts. It is evident from figs. 4 and 6 that the mean counts were much less scattered than the single counts.

Abdominal skin samples were also obtained from the first 50 patients. Three patients each had 1 positive sample which yielded 40, 120 and 40 staphylococci respectively.

In selecting the material, patients with 1,000 or more staphylococci in the axillae and perineum were excluded from further examinations. Nevertheless, two patients (Nos. 12 and 25) yielded from 2,000 to 6,000 staphylococci in samples from the axillae on the second and third examinations, and 3 patients (Nos. 9, 28 and 69) from 1,000 to 4,000 staphylococci from the perineum in one or two of the three examinations.

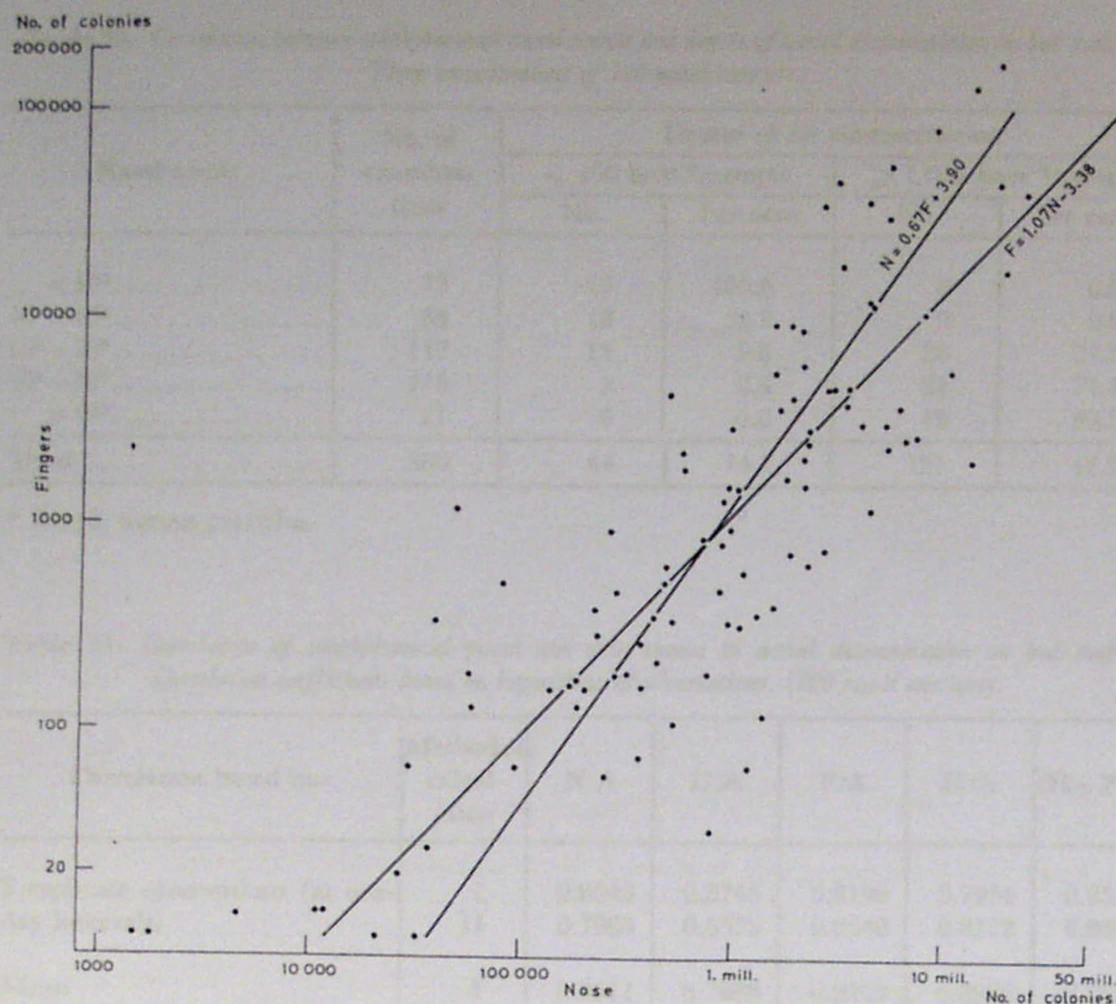

Fig. 6. Correlation between staphylococcal counts from nose and fingers (mean counts, 100 nasal carriers).

Antibiogram and phage patterns.

Antibiogram determinations and phage typing were performed on 278 colonies from the upper lip, 587 colonies from the fingers and 456 colonies from the hands. Altogether, 1,321 colonies were examined. The reactions of 1,311 colonies to the antimicrobial agents and phages were either identical with those of the respective nasal strains, or showed such slight variations that the strains were not assumed to be different. The remaining 10 colonies gave sensitivity patterns identical with the nasal strains but phage typing results were indecisive. Serological typing revealed that these strains were most probably identical.

In the 7 positive samples from the axillae and abdomen, the reactions of 8 colonies to antimicrobial agents and phages were examined. The reactions were identical with the respective nasal strains.

Antibiogram determinations and phage typing were performed on one colony from each of the positive perineal samples. One patient yielded identical strains on the perineum and in the nose, a second patient had identical strains on the perineum and in the throat and a third had a perineal strain different from that in the nose and throat.

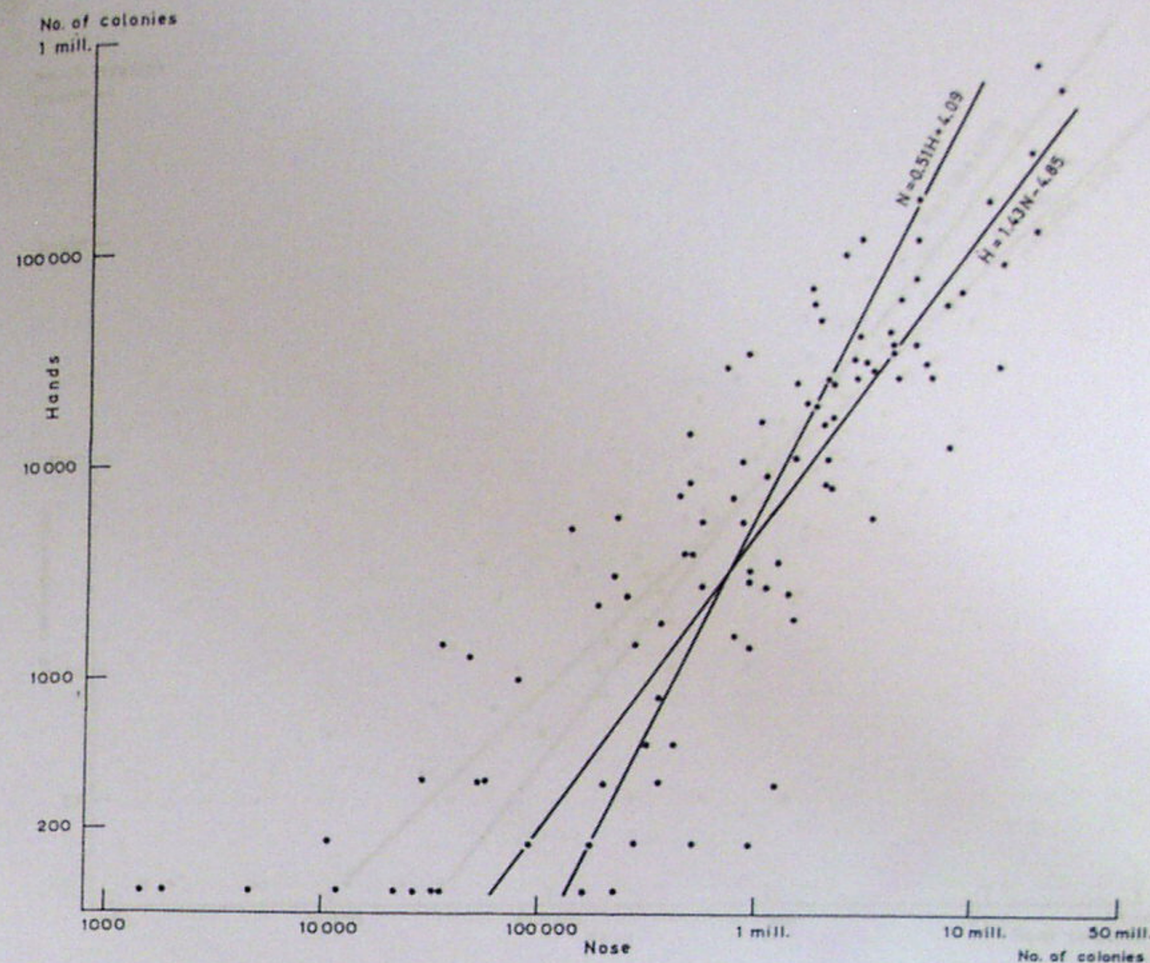

Fig. 7. Correlation between staphylococcal counts from nose and hands (mean counts, 100 nasal carriers).

e. Staphylococcal aerial dissemination on bed making.

Quantitative estimations.

The appendix table shows, for the 100 nasal carriers, the degree of staphylococcal air contamination on bed making. When staphylococci were not demonstrated in the dispersal experiments (counts less than 25), the calculation of the mean counts for the 3 examinations was based on two alternative values, 1 (method I) and 24 (method II) respectively, as mentioned in chapter II. Dispersal of staphylococci was demonstrated from 93 patients on the first examination and from 91 patients on the other examinations. The air counts varied considerably from patient to patient — from less than 25 to 43,400 Staph. aureus particles — but the day to day variations for the same patient were moderate.

The mean counts for the first, second and third examinations of the 100 patients were 2,463 (2,465), 2,446 (2,448) and 2,086 (2,088) staphylococcal particles respectively and for the 300 examinations

Table 30. *Correlation between staphylococcal nasal counts and degree of aerial dissemination on bed making. Three examinations of 100 nasal carriers.*

Nasal count	No. of examinations	Degree of air contamination			
		< 100 bact.*/sample		≥ 1,000 bact.*/sample	
		No.	Per cent	No.	Per cent
$<10^4$	13	13	100.0	0	0.0
10^4-10^5	34	18	52.9	0	0.0
10^5-10^6	117	12	9.6	26	22.2
10^6-10^7	115	1	0.9	82	71.3
$>10^7$	21	0	0.0	19	90.5
Total	300	44	14.7	127	42.3

* Staph. aureus particles.

Table 31. *Correlation of staphylococcal nasal and skin counts to aerial dissemination on bed making. Correlation coefficients based on logarithms of observations. (100 nasal carriers).*

Correlation based on:	Method of calculation	N:A	U:A	F:A	H:A	(H+F):A
3 replicate observations (at one-day intervals)	I	0.8043	0.6745	0.8199	0.7954	0.8523
	II	0.7988	0.6573	0.8340	0.8122	0.8395
Mean	I	0.8611	0.7869	0.9203	0.8986	0.9366
	II	0.8304	0.7637	0.9050	0.8797	0.9011

N = nose, U = upper lip, F = fingers, H = hands and A = air contamination.

Table 32. *Multiple correlation of combinations of mean staphylococcal nasal and skin counts to air counts. Multiple correlation coefficients based on logarithms of observations. (100 nasal carriers).*

Method of calculation	$R_{A(F+H.N)}$	$R_{A(N.U)}$	$R_{A(F+H.U)}$	$R_{A(F+H.N.U)}$
I	0.9387	0.8804	0.9378	0.9394
II	0.9120	0.8599	0.9076	0.9149

N = nose, U = upper lip, F = fingers, H = hands and A = air contamination.

$R_{y(x_1 x_2 \ldots x_n)}$ is the multiple correlation coefficient between the dependent variable y and the independent variables $x_1 \ldots x_n$.

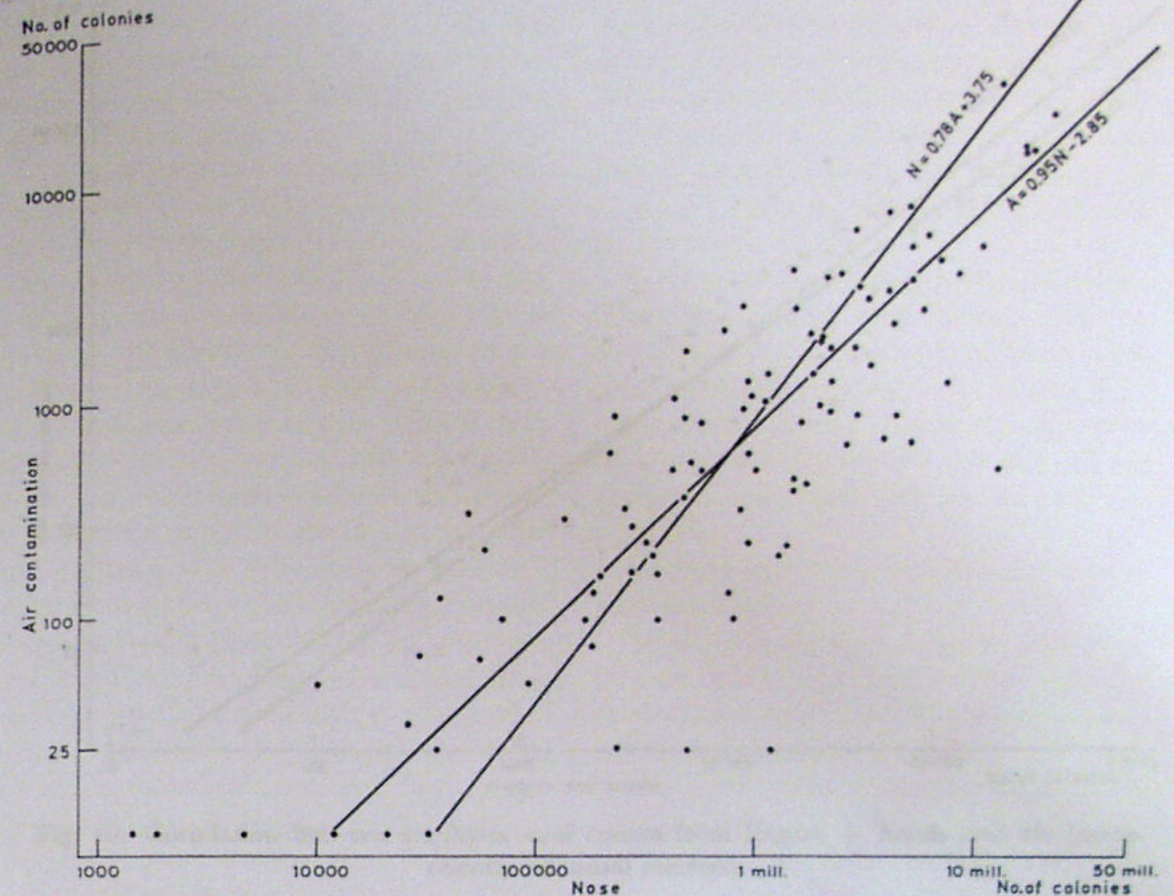

Fig. 8. Correlation between staphylococcal counts from nose and air (mean counts, 100 nasal carriers).

2,331 (2,333) particles. The figures in parenthesis are calculated on the basis of method II. The difference in the results derived from the two methods of calculation was small and the mean counts were approximately equal for the 3 examinations.

Table 30 gives the correlation between nasal and air counts on 3 examinations of the nasal carriers. Within wide limits, the air counts increased with rising numbers in nasal cultures.

The frequency distribution of the counts was found to approximate the log.-normal form. For statistical analysis, the counts were therefore transformed to a logarithmic scale and all computations made on the transformed figures.

Table 31 gives the correlation coefficients of logarithms of nasal and skin counts to air counts. The difference in the results based on the two methods of calculation was small. The correlation was least good between upper lip and air contamination, and best between fingers plus hands and air contamination.

Figs. 8—10 illustrate the correlation between mean counts from nose, fingers and fingers plus hands and mean counts of air contamination. The points marked and

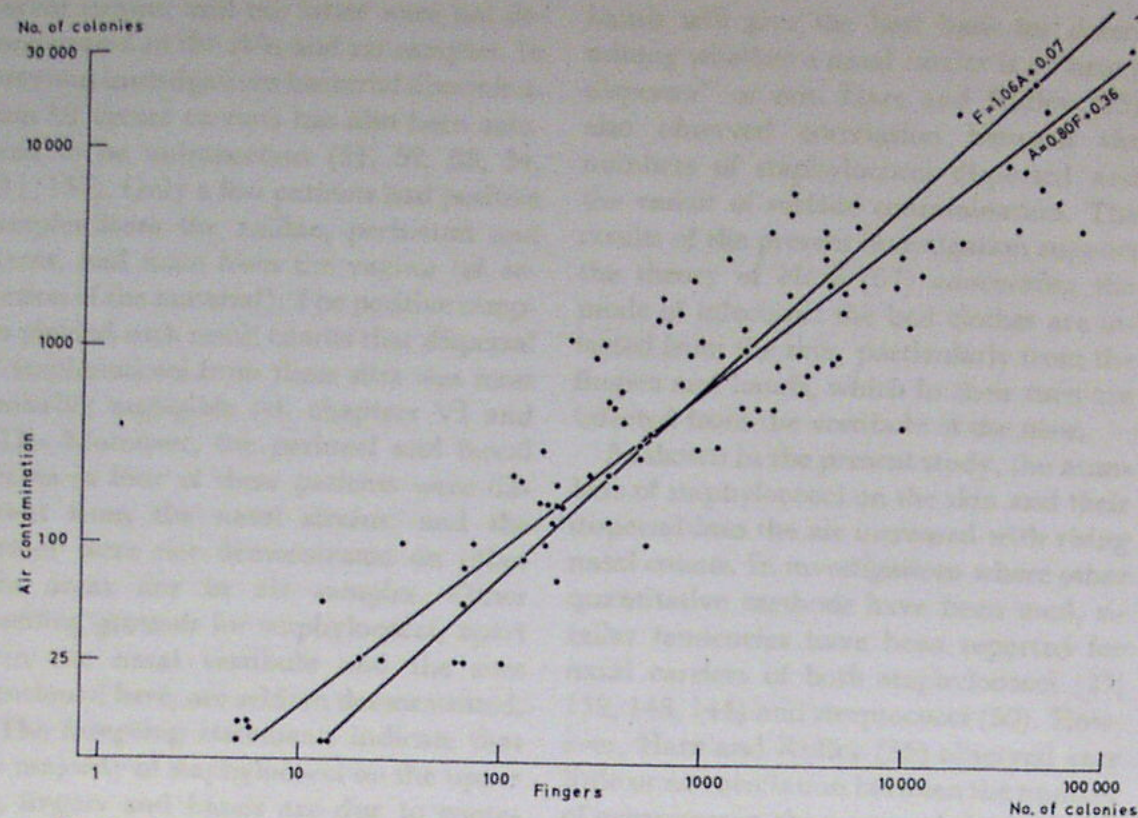

Fig. 9. Correlation between staphylococcal counts from fingers and air (mean counts, 100 nasal carriers).

the regression lines represent calculation method I. Mean counts below the lowest values on the abcissa and ordinate are plotted on the co-ordinates. It is evident from the figures that the correlation between fingers and hands combined and air contamination was better than between nose and air contamination.

Table 32 gives the multiple correlation coefficients of combinations of mean counts from nose, upper lip and fingers plus hands to mean air counts. The correlation achieved when all the combinations of measured factors which might conceivably influence air contamination were taken into consideration was only slightly better than that formerly demonstrated (table 31) between fingers plus hands and air contamination. Quantitative estimation of staphylococci in the nose and on the upper lip, after measurement of the numbers on the fingers and hands, thus provided only slight additional information concerning the air contamination.

Antibiogram and phage patterns.

Antibiogram determinations and phage typing were performed on 1,413 colonies from air samples. The reactions of 1,379 colonies to the antimicrobial agents and phages were either identical with those of the respective nasal strains, or showed such slight variations that the strains were not assumed to be different. Nine of the remaining 34 strains gave resistance patterns identical with the nasal strains, but phage typing results were indecisive. Serological

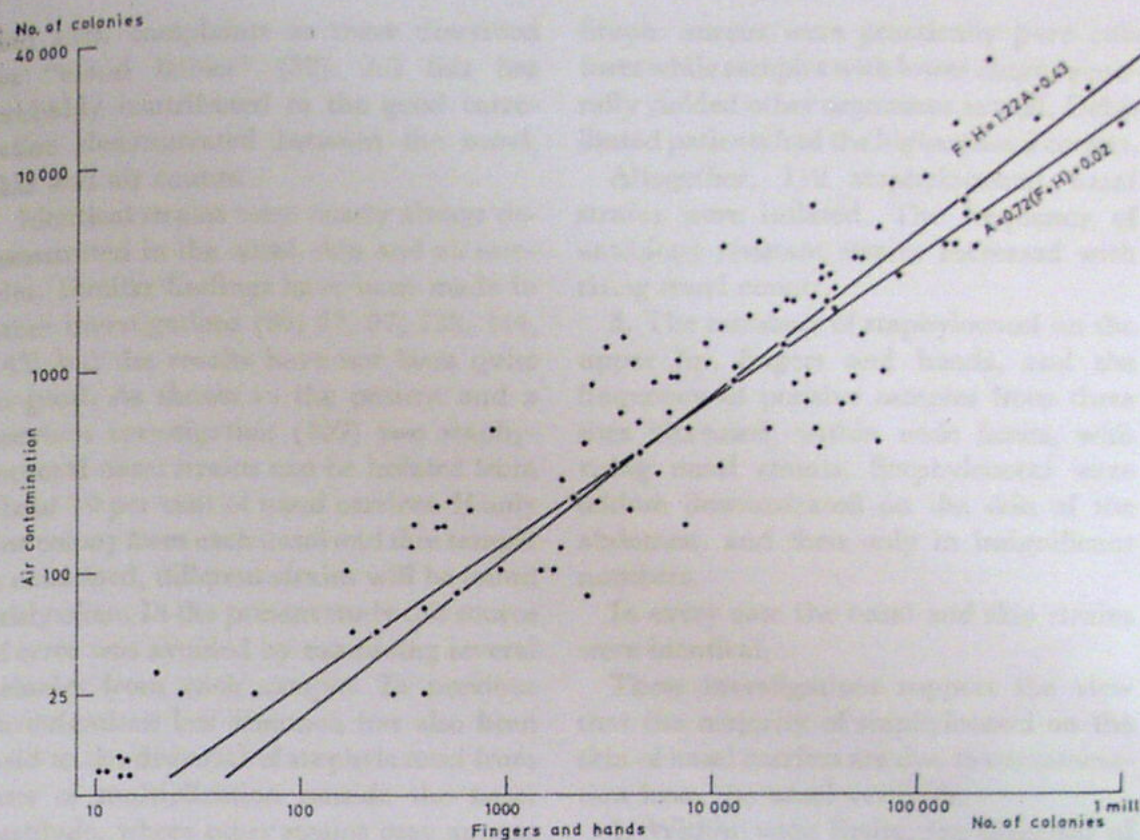

Fig. 10. Correlation between staphylococcal counts from fingers + hands and air (mean counts, 100 nasal carriers).

typing showed that in 6 of the 9 cases the air and nasal strains were identical. Consequently, 1,385 of 1,413 colonies from air samples were most probably identical with the nasal strains and 28 colonies were different.

Twenty-six of these 28 colonies were demonstrated in samples from which all staphylococcal colonies were examined, that is, in 161 samples yielding 462 colonies. The remaining 2 colonies were demonstrated in the other 139 samples in which only 951 of 6,576 staphylococcal colonies were examined. These 28 colonies were not included in the calculation of the air contamination as they were thought to be due to contamination from the environment.

3. *Discussion.*

In the present investigation an important problem was whether the staphylococci demonstrated on the various skin areas and in the air samples were really due to dispersal from the nose — directly or indirectly — and not to contamination from the surroundings or dispersal from other sites of multiplication such as the throat, vagina, perineum or faeces. As virtually all staphylococcal colonies examined in the skin and air samples were identical with the nasal strains, contamination from the surroundings must have been negligible. It is also evident that dispersal from the throat was of minor importance since 16 of the 100 patients had different nasal and

throat strains, and the latter were not demonstrated in the skin and air samples. In previous investigations bacterial dissemination by throat carriers has also been assumed to be unimportant (51, 52, 53, 54, 111, 137). Only a few patients had positive samples from the axillae, perineum and faeces, and none from the vagina (cf. selection of the material). The positive samples yielded such small counts that dispersal of staphylococci from these sites was most probably negligible (cf. chapters VI and VII). Moreover, the perineal and faecal strains in four of these patients were different from the nasal strains, and the former were not demonstrated on other skin areas nor in air samples. Other breeding grounds for staphylococci, apart from the nasal vestibule and the sites mentioned here, are seldom demonstrated.

The foregoing statements indicate that the majority of staphylococci on the upper lip, fingers and hands are due to contamination from the nose. This is also supported by the good correlation between the nasal and skin counts. In addition, very few staphylococci were demonstrated on skin areas (e. g. skin of the abdomen) which did not come into direct contact with the nasal vestibule.

The difference in the ability of nasal carriers to disperse staphylococci into the air must largely depend on the number of these organisms in the nose and on the skin. The linear correlation was better between the counts from fingers plus hands and air contamination than between the nasal and air counts. Multiple correlation analysis revealed that insignificant additional information on the ability to disperse the organisms into the air was attained by estimating the number of bacteria in the nose and on the upper lip when the counts from the fingers and hands were known. Consequently, estimation of the number of organisms on the fingers and hands will give the best basis for determining whether a nasal carrier is a "heavy disperser" or not. Hare and Ridley (55) also observed correlation between the numbers of staphylococci dispersed and the extent of surface contamination. The results of the present investigation support the theory of Hare (57) concerning the mode of infection: the bed clothes are infected from the skin, particularly from the fingers and hands, which in their turn are infected from the vestibule of the nose.

As shown in the present study, the numbers of staphylococci on the skin and their dispersal into the air increased with rising nasal counts. In investigations where other quantitative methods have been used, similar tendencies have been reported for nasal carriers of both staphylococci (77, 132, 143, 144) and streptococci (50). However, Hare and Ridley (55) observed very little or no correlation between the number of organisms in the nose and the intensity and extent of skin contamination and ability to disperse. This may be due partly to their technique which permitted only a rough estimation of the number of staphylococci in the nose and on the skin, and partly to the difficulty of maintaining a constant technique in the dispersal experiments. In the majority of studies on nasal carriers, insufficient attention has also been paid to the dispersal of staphylococci from sites of multiplication outside the nasal vestibule (faeces, perineum etc.).

On the other hand, it must be emphasized that in the present work the patients were submitted to approximately equal experimental conditions. All had bathed and received similar clean clothing and bedclothes 2 days before the first examination. They were all supplied with handkerchiefs. None were permitted to wash themselves during the last 12—14 hours before the examinations, or to get out of bed during this time. In addition, no patients had such complaints as those described for "cloud babies" (32). All this has probably contributed to the good correlation demonstrated between the nasal, skin and air counts.

Identical strains were nearly always demonstrated in the nasal, skin and air samples. Similar findings have been made in other investigations (55, 87, 97, 132, 144, 145) but the results have not been quite so good. As shown in the present and a previous investigation (109) two staphylococcal nasal strains can be isolated from about 10 per cent of nasal carriers. If only one colony from each nasal and skin sample is examined, different strains will be found fairly often. In the present study this source of error was avoided by examining several colonies from each sample. In previous investigations less attention has also been paid to the dispersal of staphylococci from sites of multiplication outside the nasal vestibule, where other strains may appear (perineum, faeces, vagina, axillae). In addition, the individuals in the present study were isolated from other staphylococcal carriers in order to prevent contamination from their surroundings.

4. *Summary and conclusions.*

1. From the patients admitted to a medical department during the course of 14 months, 100 persistent nasal carriers of Staph. aureus were selected.

In these patients the numbers of staphylococci in the nasal vestibule and on various skin sites, and the ability to disperse the organisms into the air on bed making were examined.

2. The numbers of staphylococci in the nasal cultures varied greatly from individual to individual — from less than 1,000 to several millions — but the day to day variations for the same individual were relatively small.

Nasal samples yielding more than 10^7 Staph. aureus were practically pure cultures while samples with lower counts generally yielded other organisms as well. Debilitated patients had the highest nasal counts.

Altogether, 112 staphylococcal nasal strains were isolated. The frequency of antibiotic resistant strains increased with rising nasal counts.

3. The numbers of staphylococci on the upper lip, fingers and hands, and the frequency of positive samples from these sites increased, within wide limits, with rising nasal counts. Staphylococci were seldom demonstrated on the skin of the abdomen, and then only in insignificant numbers.

In every case the nasal and skin strains were identical.

These investigations support the view that the majority of staphylococci on the skin of nasal carriers are due to contamination from the nasal vestibule.

4. Within wide limits, the dispersal of staphylococci into the air on bed making increased with rising nasal counts but there was better correlation between the number of staphylococci on the skin (fingers and hands) and aerial dissemination than between nasal and air counts.

Ninety-eight per cent of the staphylococcal colonies examined in air samples were identical with the nasal and skin strains.

The heaviest dispersers of staphylococci among nasal carriers are those individuals who yield the highest numbers of these organisms on the skin (fingers and hands). These subjects usually also have the highest numbers in nasal cultures.

5. Fifty-one of the 100 patients yielded staphylococci in throat samples and 16 had different strains in the nose and throat. In these patients the throat strains were not demonstrated in skin and air samples. Presumably throat carriers are of minor importance, compared with nasal carriers, in the dispersal of Staph. aureus.

V. Staphylococcal nasal carriers treated with framycetin-gramicidin nasal spray.

A. Previous investigations.

Nasal carriers play a considerable part in the development of staphylococcal infections. Danbolt (28) demonstrated this in the case of furunculosis as early as 1931. Later, this was shown to apply both to furunculosis (126, 130), and to other staphylococcal skin lesions (27, 35, 65, 108, 126). Perinatal pyodermias and puerperal abscesses may also be due to nosocomial infections (78, 88, 120) and postoperative septic lesions are often thought to result from autoinfection with staphylococci from the nose (23, 95, 140, 148).

Local treatment of the nasal mucosa with antibiotics can reduce the frequency of recurrent staphylococcal skin lesions among nasal carriers (27, 46, 65, 127). This treatment also seems to reduce the frequency of staphylococcal infections in maternity wards (4, 40, 72, 75, 121). In surgical wards, however, the results are not quite so uniform and encouraging (42, 43, 63, 110, 121, 140). In several investigations, other measures have been taken concurrently with antibiotic therapy and the individual effect has been difficult to assess.

Various antibiotics have been used in the treatment of staphylococcal nasal carriers and a definite reduction in nasal carriage has usually been demonstrated during and just after treatment (33, 44, 45, 129, 141). But in some investigations less favourable results have been obtained (90, 102).

In recent years, framycetin has been used alone or in combination with gramicidin in order to reduce the frequency of nasal carriers. In several investigations the results have been good (8, 71, 115, 123) but not in all (102).

The efficiency of intranasal application of antibiotics in reducing skin contamination and aerial dissemination of staphylococci by nasal carriers has seldom been studied (98, 132), and little is known of the quantitative side of the problem. The results of investigations of this aspect of the problem will be reported here.

B. Personal investigations.

1. *Material and methods.*

Nasal samples from the nurses and doctors in the department were obtained every other week for $1^1/_2$ years. Thirty individuals who had been carriers of the same staphylococcal strain for 2—6 months were selected, and the incidence and quantity of these organisms in the vestibule of the nose were determined once daily for 3 days before treatment with framycetin-gramicidin nasal spray. Nasal cultures were obtained the day after completing therapy and again at 1 week intervals.

Forty of the 100 nasal carriers in chapter IV were also treated with framycetin-gramicidin nasal spray. The numbers of staphylococci on the skin and the dispersal into the air on bed making were examined once daily for 3 days before treatment (the results are reported fully in chapter IV) and on the day after completing therapy.

Twenty of the 40 patients remained in the department for at least a further 10 days, the incidence and quantity of staphylococci in the nasal vestibule being determined on the 4th and 10th days after completing treatment. In 3 of these patients, skin and air samples were obtained several times after treatment.

In order to keep experimental conditions approximately equal before and after treatment, the 40 patients were bathed or washed on a stretcher, and received clean clothes and bedclothes 2 days before the first pre-treatment examination and 2 days before the post-treatment examination.

The framycetin-gramicidin nasal spray[1] was used 4 times daily. The spray fluid was an isotonic solution containing 1.25 per cent framycetin, 0.005 per cent gramicidin, 0.25 per cent metaoxedrin and 0.002 per cent phenylmercurinitrate. The patients were treated for 3 days and the personnel for 7 days.

The methods of investigation are described in chapter II. In assessing the frequency of nasal carriers, nasal cultures which did not yield staphylococci in the first dilution (1 : 40) were regarded as negative.

2. *Results.*

Tables 33 and 34 give the frequency of nasal carriers of Staph. aureus among the personnel and patients after treatment. The frequency was lowest for the personnel who had been treated longest, and in both groups it was lowest on the day after completion of treatment and rose rapidly in the course of the following 1—2 weeks. Antibiogram determinations and phage typing were performed on 1—2 colonies from all positive nasal cultures. Only 3 individuals in each group yielded different strains after therapy.

Figs. 11 and 12 illustrate the mean nasal counts from the personnel and patients before and after treatment. In both groups, there was a marked reduction from high pre-treatment values to less than 0.01 per cent of the original numbers the day after completion of therapy. The nasal counts from the majority of individuals in both

[1] The preparation was supplied by "Nyco", Oslo.

Table 33. *Staphylococcal nasal carriage after treatment with framycetin-gramicidin nasal spray for seven days.*
(30 members of the personnel).

Day after treatment	No. of samples		Per cent of samples positive
	Positive	Negative	
1	3	27	10
7	17	13	57
14	23	7	77
21	25	5	81
28	27	3	90

Table 34. *Staphylococcal nasal carriage after treatment with framycetin-gramicidin nasal spray for three days.*
(20 patients).

Day after treatment	No. of samples		Per cent of samples positive
	Positive	Negative	
1	8	12	40
4	11	9	55
10	18	2	90

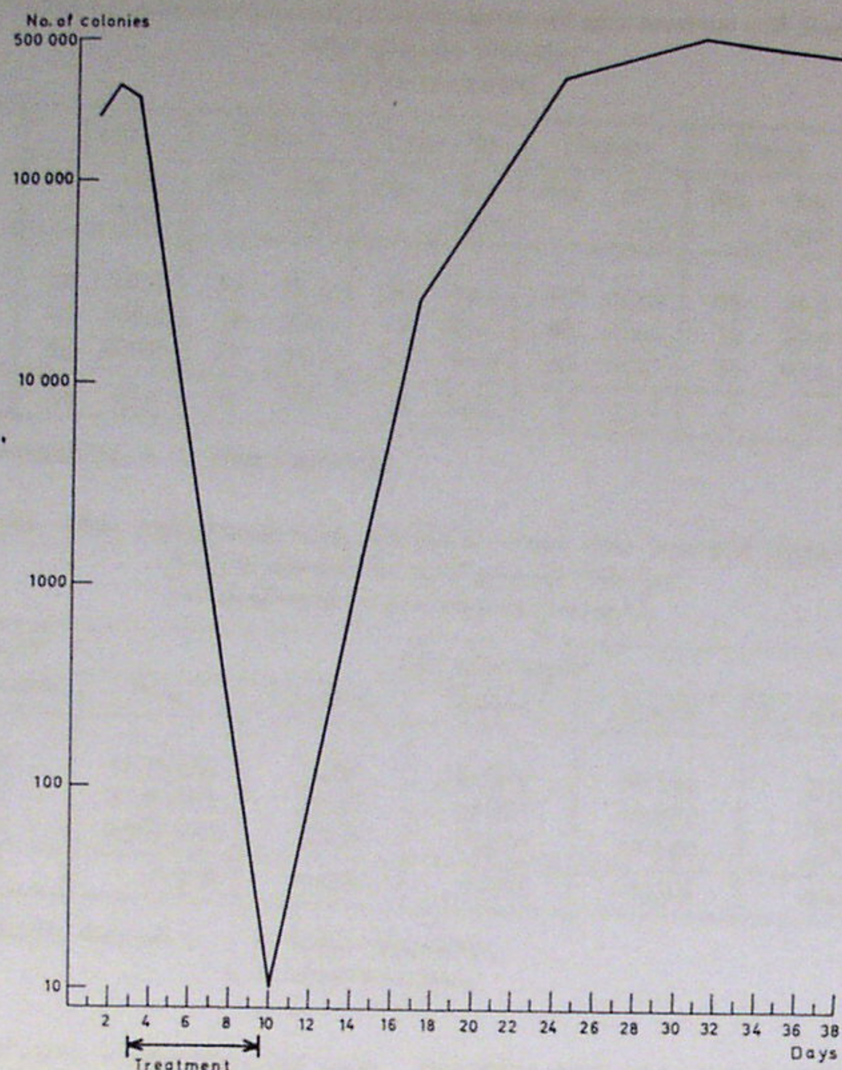

Fig. 11. Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 30 nasal carriers).

groups were still low 4 and 7 days later, but during the subsequent week the mean counts for both groups rose to about the same level as before treatment.

Quantitative nasal cultures were obtained for 10 days from 10 untreated subjects (patients and personnel) who had been shown by weekly examinations (covering 1 to 2 months) to be persistent staphylococcal nasal carriers. Fig. 13 illustrates the mean numbers of Staph. aureus in daily samples from these individuals. Only minor variations were observed.

Table 35 gives the frequency of staphylococcal positive nasal, throat, skin and air samples from 40 nasal carriers before and after treatment. From the throat, staphylococci were isolated almost as frequently after treatment as before, but in the other samples the organisms were seldom demonstrated after treatment.

Table 36 and figs. 14 and 15 give the mean counts in nasal, skin and air samples

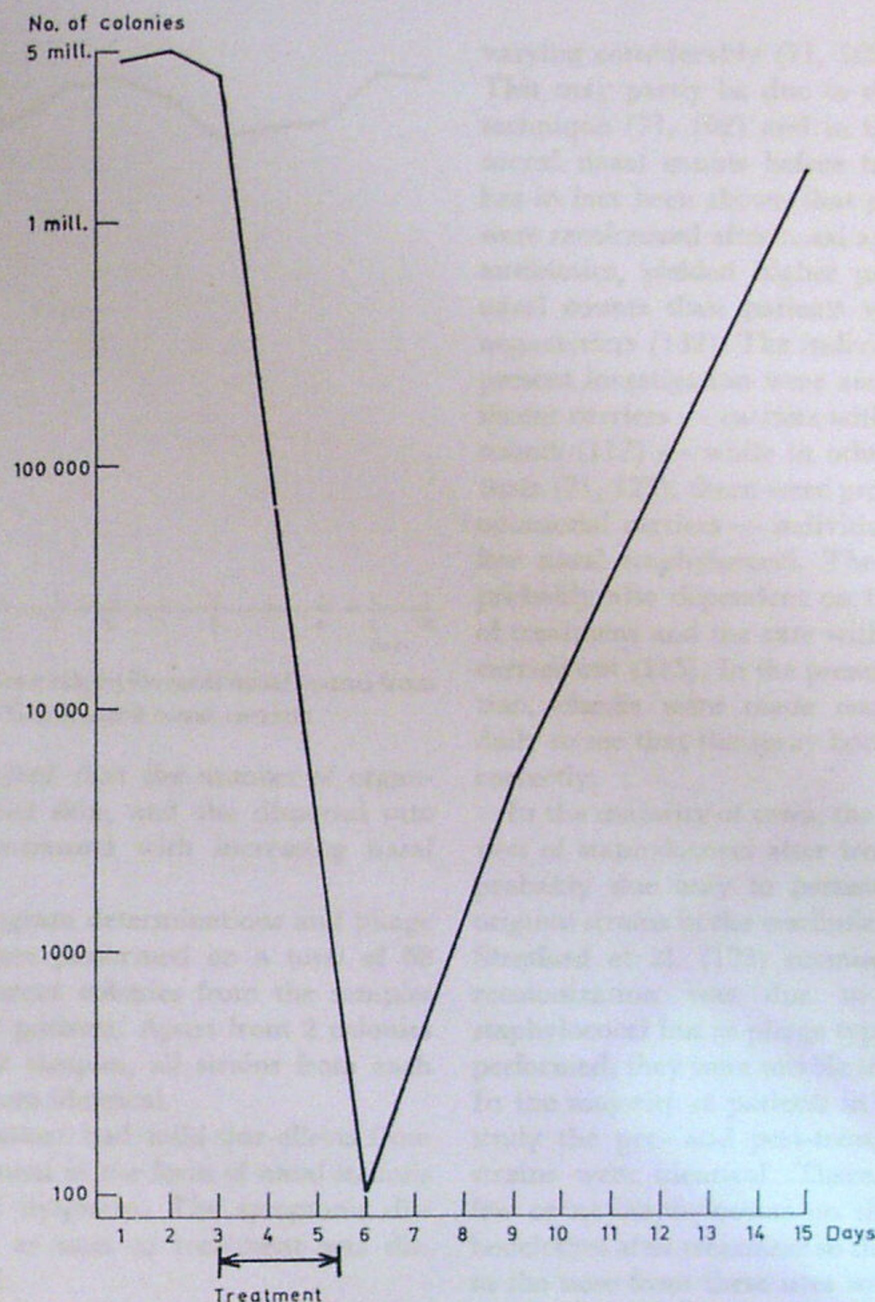

Fig. 12. Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 20 nasal carriers).

from 40 nasal carriers before and after treatment. For all samples, a marked reduction was demonstrated from high values before treatment to extremely low values the day after treatment was completed.

Three patients had positive perineal samples (from 2,000 to 8,000 staphylococci) the day after completing treatment.

Sixteen of the 40 patients yielded positive post-treatment nasal cultures. Antibiogram determinations and phage typing were performed on 18 colonies from these

Table 35. *Frequency of staphylococcal positive samples before and after treatment with framycetin-gramicidin nasal spray for three days. (40 nasal carriers).*

Day of treatment	Nose		Throat		Upper lip		Fingers		Hands		Air contam.	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
−3	40	100.0	13	32.5	36	90.0	40	100.0	39	97.5	40	100.0
−2	40	100.0	12	30.0	35	87.5	40	100.0	39	97.5	40	100.0
−1	40	100.0	11	27.5	37	92.5	40	100.0	39	97.5	40	100.0
+1	16	40.0	8	20.0	8	20.0	5	12.5	1	2.5	9	22.5

− = before treatment, + = after treatment.

Table 36. *Mean staphylococcal nasal, skin and air counts before and after treatment with framycetin-gramicidin nasal spray for three days. (40 nasal carriers, mean counts in thousands).*

Day of treatment	No. of bacteria*				
	Nose	Upper lip	Fingers	Hands	Air contam.
−3	4,614.650	2.380	13.925	64.163	4.280
−2	3,746.500	3.182	13.986	51.263	4.433
−1	4,600.080	2.302	17.291	57.100	3.888
+1	0.155	0.009	0.003	0.013	0.012

* Calculation method I, − = before treatment, + = after treatment.

samples. In all cases, identical strains were demonstrated before and after treatment. Altogether, 25 staphylococcal colonies were demonstrated in the 14 positive samples from upper lip, fingers and hands of the 40 patients after treatment. Antibiogram determinations and phage typing were performed on 15 colonies. Only 1 colony differed from the corresponding nasal strain. Seventeen Staph. aureus colonies were demonstrated in the air samples after treatment. Seven colonies were different from the respective nasal strains. Skin and air sample colonies which differed from the respective nasal strains were assumed to be due to environmental contamination and were not included in the calculations of skin and air contamination.

Eight patients had positive throat samples after treatment. Antibiogram determinations and phage typing were performed on 10 colonies from these samples. Five patients had identical throat strains before and after therapy. Staphylococci were not demonstrated in skin and air samples from 3 patients who yielded positive throat cultures and negative nasal cultures after treatment.

Three patients were examined several times after completing treatment with nasal spray. Table 37 shows the results of these post-treatment examinations, which

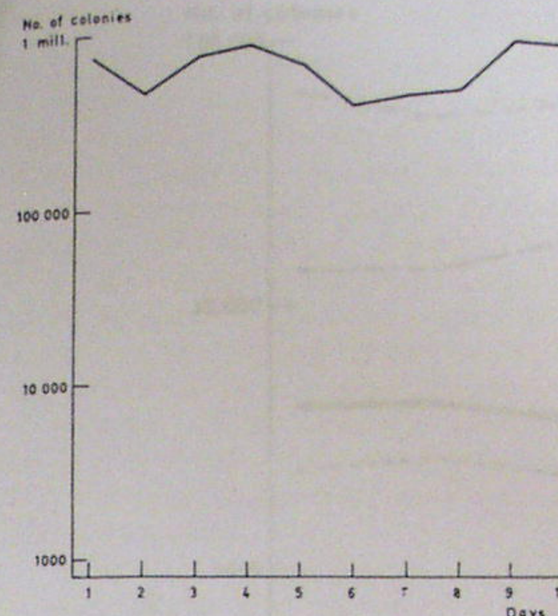

Fig. 13. Mean staphylococcal nasal counts from 10 untreated nasal carriers.

demonstrated that the number of organisms on the skin, and the dispersal into the air increased with increasing nasal counts.

Antibiogram determinations and phage typing were performed on a total of 68 Staph. aureus colonies from the samples of these 3 patients. Apart from 2 colonies in the air samples, all strains from each patient were identical.

One patient had mild side-effects from the treatment in the form of nasal stenosis and mild dyspnoea. The symptoms disappeared as soon as treatment was discontinued.

Staphylococcal strains resistant to framycetin and gramicidin were not observed during the investigation.

3. *Discussion.*

Nasal spray therapy has only a temporary influence on the carrier status. Staphylococci are often demonstrated after the completion of treatment, the interval before the nasal samples become positive varying considerably (71, 102, 115, 123). This may partly be due to differences in technique (71, 102) and in the staphylococcal nasal counts before treatment. It has in fact been shown that patients who were recolonized after nasal application of antibiotics, yielded higher pre-treatment nasal counts than patients who became non-carriers (132). The individuals in the present investigation were accounted persistent carriers — carriers with high nasal counts (117) — while in other investigations (71, 123), there were probably more occasional carriers — individuals yielding few nasal staphylococci. The results are probably also dependent on the duration of treatment and the care with which it is carried out (115). In the present investigation, checks were made once or twice daily to see that the spray bottle was used correctly.

In the majority of cases, the demonstration of staphylococci after treatment was probably due only to persistence of the original strains in the vestibule of the nose. Stratford et al. (123) surmised that the recolonization was due to exogenous staphylococci but as phage typing was not performed, they were unable to prove this. In the majority of patients in the present study the pre- and post-treatment nasal strains were identical. There were very few or no staphylococci on the skin and bedclothes after treatment so that dispersal to the nose from these sites was most unlikely. Re-infection from the throat was, on the other hand, possible in some cases. In the majority, however, persistence of the original organisms in the vestibule of the nose was the most reasonable explanation. This is in accordance with the results of other investigations (71, 102).

One of the 3 individuals in the personnel group who changed strains had negative nasal cultures for 22 days, but the day after

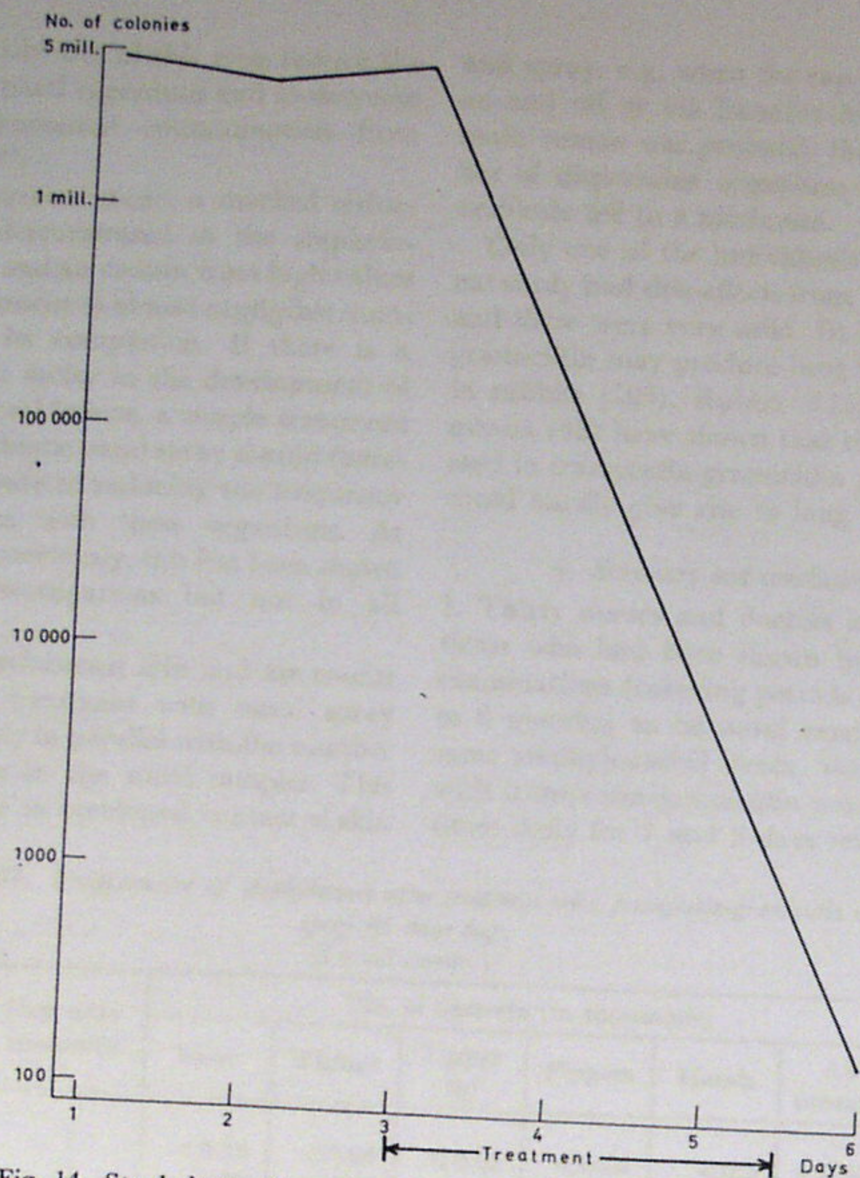

Fig. 14. Staphylococcal nasal counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 40 nasal carriers).

she began nursing a patient isolated for a severe staphylococcal pyodermia (patient 1, chapter VII), her nasal cultures yielded a strain identical with that harboured by the patient.

In one of the 3 patients who changed strains, the one developed in the nose after treatment was identical with the strain yielded by the perineum. Another of these 3 patients, who was placed in the same room as a perineal carrier who dispersed large numbers of staphylococci, yielded after 3 days positive nasal cultures of a strain identical with that of the perineal carrier.

Routine examination of air contamination in the wards and charting of all staphylococcal carriers among personnel and patients showed that those individuals who changed their strains, were recolonized

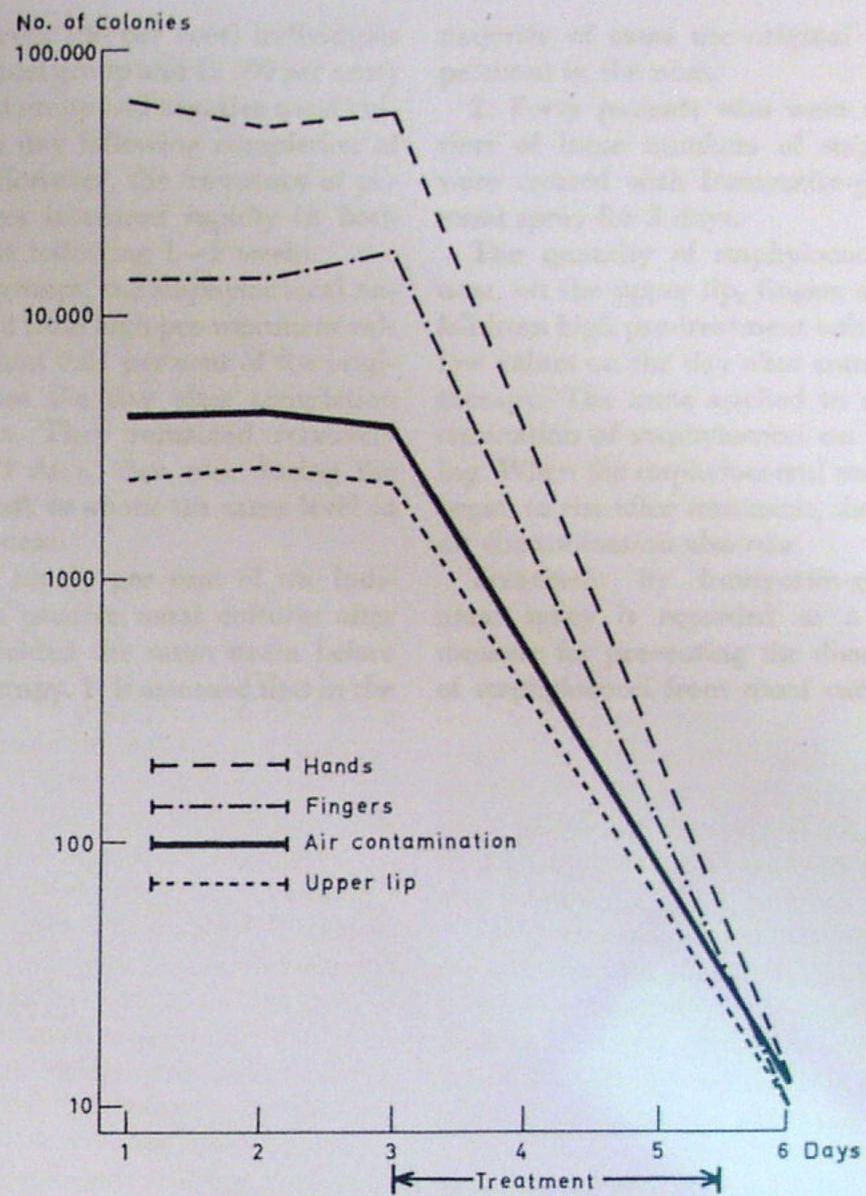

Fig. 15. Staphylococcal skin and air counts before and after treatment with framycetin-gramicidin nasal spray (mean counts, 40 nasal carriers).

with those organisms which appeared in greatest numbers. These observations are few in number, but there is probably a quantitative factor in the transfer of staphylococci from one individual to another.

Although staphylococci can be demonstrated in nasal cultures after treatment, a purely qualitative assessment does not give a true picture of the effect of treatment. Porter et al. (102) did not undertake quantitative investigations but they were nevertheless aware of this: "Although the use of framycetin seemed to have little effect on the over-all pattern of staphylococcal nasal carriage it may be that

topical antibiotics of this type reduce the density of nasal organisms and so decrease the environmental contamination from this source".

In the present study, a marked reduction was demonstrated in the staphylococcal skin and air counts, from high values before treatment to almost negligible numbers after its completion. If there is a quantitative factor in the development of staphylococcal lesions, a simple treatment such as antibiotic nasal spray should therefore contribute to reducing the frequency of infections with these organisms. As mentioned previously, this has been shown in some investigations but not in all (63, 121).

The staphylococcal skin and air counts fell during treatment with nasal spray approximately in parallel with the number of organisms in the nasal samples. This might be due to accidental contact of skin and spray, e.g. when the cap was screwed on and off or via handkerchiefs, but the main reason was probably that the number of dispersable organisms in the nasal vestibule fell to a minimum.

Only one of the individuals in the present study had side-effects from the therapy and these were very mild. In large doses, gramicidin may produce lung infiltrations in rabbits (105). Rubbo (113) and Gremeaux (48) have shown that the quantity used in framycetin-gramicidin nasal spray could hardly give rise to lung symptoms.

4. *Summary and conclusions.*

1. Thirty nurses and doctors and 20 patients who had been shown by repeated examinations (covering periods of 3 weeks to 6 months) to be nasal carriers of the same staphylococcal strain, were treated with framycetin-gramicidin nasal spray 4 times daily for 7 and 3 days respectively.

Table 37. *Reappearance of staphylococci after treatment with framycetin-gramicidin nasal spray for three days.*
(3 nasal carriers).

Pat. no.	Day after treatment	No. of bacteria (in thousands)					
		Nose	Throat	Upper lip	Fingers	Hands	Air contam.
1	1	<0.04	<0.04	<0.02	<0.02	<0.5	<0.025
	2	1.60	<0.04	0.02	0.02	<0.5	0.025
	3	14.00	<0.04	0.02	0.02	<0.5	0.025
	6	84.00	0.04	0.44	0.08	4.0	<0.025
	7	184.00	<0.04	0.44	0.72	11.0	0.300
2	4	17.00	<0.04	<0.02	<0.02	<0.5	0.025
	5	36.00	<0.04	0.02	<0.02	<0.5	<0.025
	6	57.00	<0.04	<0.02	0.18	<0.5	0.100
	7	252.00	<0.04	<0.02	0.12	<0.5	0.300
	11	2,400.00	<0.04	5.60	3.60	12.0	4.900
3	1	<0.04	<0.04	<0.02	<0.02	<0.5	<0.025
	3	0.16	<0.04	<0.02	<0.02	<0.5	<0.025
	29	1,600.00	0.84	0.64	3.92	20.0	1.200
	30	800.00	0.36	0.40	1.78	1.5	0.300

Twenty-seven (90 per cent) individuals in the personnel group and 12 (60 per cent) in the patient group had negative nasal cultures on the day following completion of treatment. However, the frequency of positive cultures increased rapidly in both groups in the following 1—2 weeks.

For both groups, the staphylococcal nasal counts fell from high pre-treatment values to less than 0.01 per cent of the original counts on the day after completion of treatment. They remained relatively low for 4—7 days, then rose during the following week to about the same level as before treatment.

Eighty to ninety per cent of the individuals with positive nasal cultures after treatment yielded the same strain before and after therapy. It is assumed that in the majority of cases the original organisms persisted in the nose.

2. Forty patients who were nasal carriers of large numbers of staphylococci were treated with framycetin-gramicidin nasal spray for 3 days.

The quantity of staphylococci in the nose, on the upper lip, fingers and hands fell from high pre-treatment values to very low values on the day after completion of therapy. The same applied to aerial dissemination of staphylococci on bed making. When the staphylococcal nasal counts began to rise after treatment, the skin and air contamination also rose.

Treatment by framycetin-gramicidin nasal spray is regarded as a valuable measure for preventing the dissemination of staphylococci from nasal carriers.

VI. Perineal carriers of Staph. aureus examined before and after treatment with hexachlorophane.

A. Previous investigations.

The perineum was first suggested as a potential source and breeding-place of Staph. aureus by Hare and Ridley (55). Gillespie et al. (41) isolated Staph. aureus from the perineum of 30 to 50 per cent of 2 to 10 day-old neonates. Ridley (107) obtained sufficient numbers of pathogenic staphylococci from the perineal area of 11 of 50 male students to class them as perineal carriers, 10 per cent having large numbers of these organisms on the perineum and very few of no nasal staphylococci. Tulloch et al. (127) examined the perineum of patients suffering from chronic furunculosis: 15 of 24 patients were perineal carriers of staphylococci. They suggested that this figure was probably higher than in the general population because perineal swabs were initially obtained from patients with boils on the lower part of the body. Bøe et al. (19) examined 3,508 patients admitted to a medical ward. Thirteen per cent had Staph. aureus on the perineum, either alone (3 per cent) or combined with nasal and throat carriage.

The dispersal of staphylococci from perineal carriers has seldom been investigated (56, 57, 107). Perineal carriers, like nasal carriers, differ remarkably in their ability to disperse these organisms (57, 107) but the reason for this is unknown.

On limited data it has been suggested that combined nasal and perineal carriers are liable to disperse larger numbers of staphylococci than pure nasal carriers (57, 107). Attempts to reduce the dissemination of Staph. aureus by perineal carriers have not been made.

In the present study, the difference in ability of perineal carriers to disperse staphylococci was investigated. The possibility of reducing the number of staphylococci on the skin, and their dispersal into the air by hexachlorophane (pHaisoHex*) skin disinfection was also examined.

B. Personal investigations.

1. *Material and methods.*

The material, comprising 5 women and 9 men between 9 and 64 years of age, was obtained in the following way. All patients (2,014 in all) admitted to The Medical Department B from August 1962 to October 1963 were examined for Staph. aureus in the nose, throat and on the perineum. About 13 per cent of the patients were perineal carriers on admission and these were examined for perineal staphylococci 4 times at intervals of 2—3 days. At the final examination, the numbers of these organisms in the nose, axillae, vagina and faeces were also estimated.

Patients yielding perineal staphylococci on all 4 examinations were regarded as predominantly perineal carriers, provided that they did not have staphylococcal lesions, more than 1,000 Staph. aureus in the samples from axillae, vagina and faeces, and more than 1 million Staph. aureus in nasal samples. These criteria will be evident from the investigations referred to in chapters IV and VII. Fifteen patients fulfilled the conditions but 1 of them was too ill to be examined further.

The remaining 14 patients were examined 1—5 times in the course of 1—7 days. One patient (No. 13) was examined on 2 separate admissions 5 months apart.

Five of the 14 patients (Nos. 7, 11, 12, 13 and 14) were then treated morning and evening for 3 days by washing the perineum with 3 per cent hexachlorophane emulsion. Further samples were obtained the morning after completing treatment. One patient (No. 14) received treatment for a further 3 days and was re-examined the next morning. Patient 13 was treated on both admissions to hospital.

The patients themselves undertook the treatment after preliminary instruction. The skin was first moistened with water. Hexachlorophane emulsion was then rubbed over the perineum and adjacent regions and washed off after 1 minute. The procedure was supervised to ensure correct technique. Other washing was performed with non-disinfectant soap.

In order to establish approximately equal experimental conditions before and after treatment, the patients were bathed and received clean clothes and bedclothes 2 days before the first pre-treatment examination and 2 days before the post-treatment examination.

The methods of investigation are described in chapter II. All sampling fluids and media after treatment contained 1 per cent "Tween 80" to neutralize any residues of hexachlorophane emulsion that might be present.

2. *Results.*

Quantitative estimations.

The results of the examinations before treatment are given in table 38. The patients were divided into 3 groups according to the number of staphylococci on the perineum. Group 1 yielded less than 10^4 Staph. aureus in these samples, group 2 between 10^4 and 10^6 and group 3 more than 10^6 Staph. aureus.

Tables 39—42 give the correlation between perineal counts and counts from upper lip, fingers, hands and the skin just outside the perineum, respectively. Within wide limits, the numbers of staphylococci in these samples increased with rising perineal counts.

Table 43 gives the correlation between the perineal counts and the staphylococcal aerial dissemination on bed making. It is evident that the ability to disperse the organisms into the air also increased with rising numbers on the perineum.

Five heavy dispersers were treated with hexachlorophane skin disinfection. Four of them showed a marked reduction of skin counts and aerial dissemination of staphylococci, from high pre-treatment values to negligible values the day after completion of treatment. The results of the examinations of one of them (patient 13, second hospital admission) given in fig. 16, provide a further illustration of this.

The fifth patient (No. 14) also showed a marked reduction in the number of staphylococci during treatment, although the samples still yielded a few organisms

*) Manufactured by The Winthrop Products Company, Surbiton, England.

Table 38. *Skin contamination and aerial dissemination of staphylococci; 14 perineal carriers. No. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Day of examin.	Perineum	Nose	Throat	Upper lip	Fingers	Hands	Extra-perineal area	Air contamination (bed making)
1 F 26	1	4	80.00	0.04	<0.02	<0.02	<0.5	<0.08	<0.025
	2	1	92.00	<0.04	<0.02	<0.02	<0.5	<0.08	<0.025
2 M 23	1	2	0.04	<0.04	<0.02	0.04	<0.5	<0.08	<0.025
	2	9	0.08	<0.04	<0.02	<0.02	<0.5	<0.08	<0.025
3 M 21	1	2	48.00	<0.04	0.04	<0.02	<0.5	<0.08	0.100
	2	6	28.00	<0.04	0.02	0.02	0.5	<0.08	0.100
4 M 16	1	2	4.00	0.08	<0.02	0.04	<0.5	0.08	0.100
	2	3	20.00	<0.04	<0.02	0.04	<0.5	0.40	0.100
5 F 46	1	80	<0.04	0.04	<0.02	<0.02	<0.5	0.16	3.300
	2	280	<0.04	<0.04	0.02	<0.02	1.0	0.08	2.500
	5	44	<0.04	<0.04	<0.02	0.02	<0.5	<0.08	0.900
6 F 53	1	444	120.00	<0.04	<0.02	0.02	<0.5	1.68	1.800
	4	960	148.00	<0.04	<0.02	0.14	<0.5	<0.08	1.500
	5	400	16.00	<0.04	<0.02	<0.02	<0.5	4.00	0.200
7 F 49	1	464	1.04	<0.04	<0.02	0.18	2.5	44.00	3.000
	6	920	0.16	0.04	0.08	4.56	40.0	4.00	2.400
	7	160	<0.04	<0.04	<0.02	0.18	6.0	6.00	1.700
8 F 10	1	351	16.00	1.52	<0.02	4.18	18.0	1.04	3.700
	3	891	4.00	3.42	1.54	10.56	105.0	0.88	35.400
9 M 33	1	8	<0.04	<0.04	<0.02	<0.02	<0.5	<0.08	<0.025
	4	440	20.00	<0.04	0.02	4.12	12.0	0.32	13.900
10 M 27	1	3	<0.04	<0.04	<0.02	<0.02	<0.5	<0.08	<0.025
	5	252	0.04	<0.04	<0.02	2.82	11.5	2.40	7.900

Table 38 cont. *Skin contamination and aerial dissemination of staphylococci; 14 perineal carriers. No. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Day of examin.	Perineum	Nose	Throat	Upper lip	Fingers	Hands	Extra-perineal area	Air contamination (bed making)
11 M 65	1	640	0.04	<0.04	<0.02	7.82	26.5	16.00	24.700
12 M 23	1	1,000	12.00	0.76	<0.02	0.96	84.0	7.00	1.300
	3	2,560	16.00	0.88	<0.02	26.80	30.0	3.00	16.700
	4	1,840	16.00	0.60	<0.02	9.68	16.5	16.00	14.100
13 M 14	1	2,240	20.00	<0.04	1.48	53.00	210.0	36.00	51.900
	2	8,160	149.00	0.04	5.28	33.00	66.0	68.00	56.900
	3	4,320	61.00	<0.04	0.40	11.00	120.5	104.00	52.300
	1	18,200	12.00	0.04	1.24	287.00	1,710.0	64.00	111.200
	2	15,720	4.00	<0.04	8.48	126.00	360.0	424.00	114.800
	3	26,520	8.00	<0.04	1.56	670.00	1,320.0	920.00	210.400
	4	12,400	12.00	<0.04	0.36	128.00	420.0	528.00	144.900
	5	11,280	16.00	<0.04	6.48	51.00	840.0	160.00	131.800
14 M 60	1	10,720	64.00	<0.04	0.08	64.00	180.0	36.00	260.400
	2	24,400	160.00	<0.04	1.08	61.00	730.0	21.00	206.700

Table 39. *Correlation between staphylococcal counts from perineum and upper lip. 14 perineal carriers.*

Perineal count	No. of examinations	Upper lip			
		<20 bact./sample		>1,000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$....	10	8	80.0	0	0.0
10^4-10^6....	14	10	71.4	1	7.1
$>10^6$....	13	3	23.1	7	53.8
Total	37	21	56.8	8	21.6

Table 40. *Correlation between staphylococcal counts from perineum and fingers. 14 perineal carriers.*

Perineal count	No. of examinations	Fingers			
		<20 bact./sample		>1,000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$....	10	6	60.0	0	0.0
10^4-10^6....	14	3	21.4	6	42.9
$>10^6$....	13	0	0.0	12	92.3
Total	37	9	24.3	18	48.6

Table 41. *Correlation between staphylococcal counts from perineum and hands. 14 perineal carriers.*

Perineal count	No. of examinations	Hands			
		<500 bact./sample		>10,000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$....	10	9	90.0	0	0.0
10^4-10^6....	14	5	35.7	3	21.4
$>10^6$....	13	0	0.0	13	100.0
Total	37	14	37.8	16	43.2

Table 42. *Correlation between staphylococcal counts from perineum and extra-perineal area. 14 perineal carriers.*

Perineal count	No. of examinations	Extra-perineal area			
		<80 bact./sample		>10,000 bact./sample	
		No.	Per cent	No.	Per cent
$<10^4$....	10	8	80.0	0	0.0
10^4-10^6....	14	2	14.3	2	14.3
$>10^6$....	13	0	0.0	10	76.9
Total	37	10	27.0	12	32.4

Table 43. *Correlation between staphylococcal perineal counts and degree of aerial dissemination on bed making. 14 perineal carriers.*

Perineal count	No. of examinations	Degree of air contamination			
		<100 bact.*/sample		>10,000 bact.*/sample	
		No.	Per cent	No.	Per cent
$<10^4$....	10	6	60.0	0	0.0
10^4-10^6....	14	0	0.0	3	21.4
$>10^6$....	13	0	0.0	12	92.3
Total	37	6	16.2	15	40.5

* Staph. aureus particles.

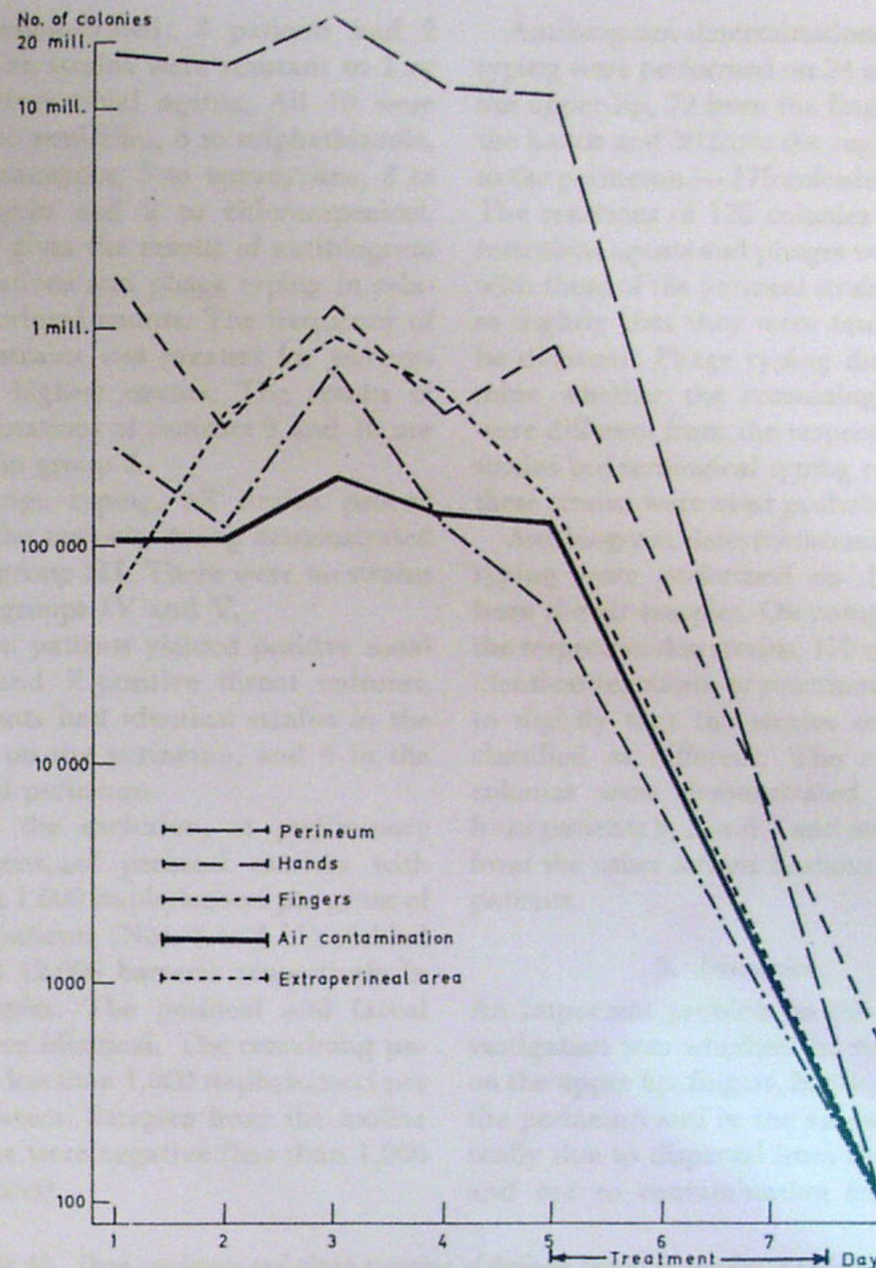

Fig. 16. Staphylococcal counts from patient 13 before and after treatment with hexachlorophane emulsion.

(fig. 17). The patient was treated for a further 3 days after which, samples from the perineum and adjacent area, and from the fingers and hands, no longer yielded Staph. aureus and only 20 bacteria were isolated from the upper lip. The aerial dissemination was also minimal (100 bacteria).

Patient 13 yielded no staphylococci in perineal cultures on discharge after the first stay in hospital but, as seen in table 38, larger quantities were demonstrated 5

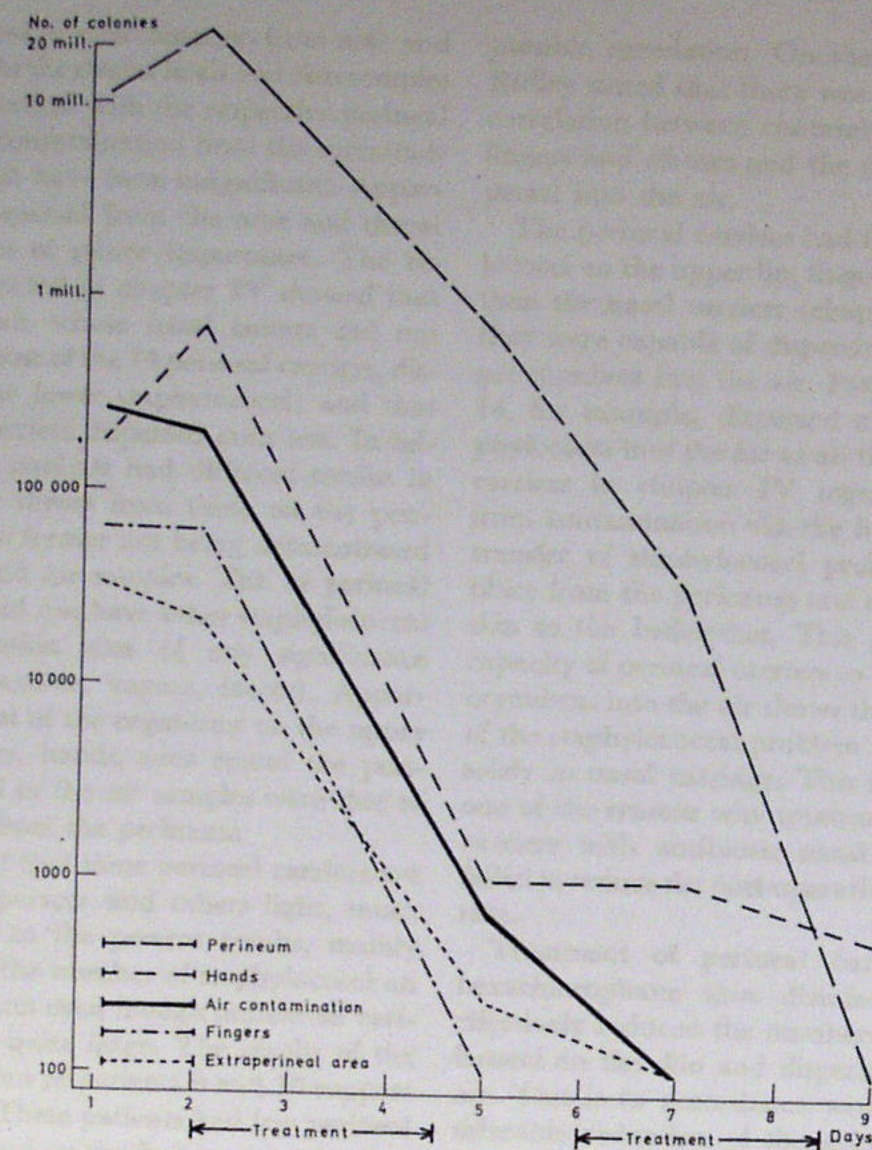

Fig. 17. Staphylococcal counts from patient 14 before, during and after treatment with hexachlorophane emulsion.

months later than at the first admission. The strains were identical on both occasions. The perineal count from patient 14 was small the day after completion of treatment but increased during the following 7 days, the strain being identical with that previously demonstrated.

The number of staphylococci in the vestibule of the nose was unchanged by the treatment.

No side-effects were observed. On the other hand, 2 patients (Nos. 13 and 14) suffered from perineal pruritus which disappeared during treatment.

Antibiogram and phage patterns.

On the average, antibiogram determinations and phage typing were performed on 5 perineal colonies from each patient. Sixty-nine colonies were examined and 17 strains demonstrated: 3 patients had 2 strains. Ten strains were resistant to 1 or more antimicrobial agents. All 10 were resistant to penicillin, 6 to sulphathiazole, 5 to streptomycin, 5 to tetracycline, 3 to erythromycin and 2 to chlorampenicol. Table 44 gives the results of antibiogram determinations and phage typing in relation to perineal counts. The frequency of resistant strains was greatest for patients with the highest counts. The results of the examinations of patients 9 and 10 are included in group 2.

On phage typing, all strains proved typable, the majority being demonstrated in phage group III. There were no strains in phage groups IV and V.

Thirteen patients yielded positive nasal cultures and 7 positive throat cultures. Ten patients had identical strains in the nose and on the perineum, and 4 in the throat and perineum.

Despite the exclusion, at preliminary examinations, of perineal carriers with more than 1,000 staphylococci per gram of faeces, 2 patients (Nos. 3 and 11) yielded 1,000 and 12,000 bacteria respectively in these samples. The perineal and faecal strains were identical. The remaining patients had less than 1,000 staphylococci per gram of faeces. Samples from the axillae and vagina were negative (less than 1,000 staphylococci).

Antibiogram determinations and phage typing were performed on 24 colonies from the upper lip, 72 from the fingers, 49 from the hands and 30 from the region adjacent to the perineum — 175 colonies altogether. The reactions of 172 colonies to the antimicrobial agents and phages were identical with those of the perineal strains, or varied so slightly that they were assumed not to be different. Phage typing did not determine whether the remaining 3 colonies were different from the respective perineal strains but serological typing revealed that these strains were most probably identical.

Antibiogram determinations and phage typing were performed on 173 colonies from the air samples. On comparison with the respective skin strains, 170 colonies gave identical reactions, or reactions that varied so slightly that the strains could not be classified as different. The remaining 3 colonies were demonstrated in samples from patients 3, 5 and 7 and were different from the other strains harboured by these patients.

3. *Discussion.*

An important problem in the present investigation was whether the staphylococci on the upper lip, fingers, hands, area round the perineum and in the air samples were really due to dispersal from the perineum and not to contamination from the en-

Table 44. *Drug sensitivity and phage grouping of perineal strains in relation to perineal count.*

Perineal count	No. of strains					
	Total	Resistant	Phage groups			
			I	II	III	Miscellaneous
$<10^4$	5	2	2	1	1	1
10^4-10^6	8	5	1	0	6	1
$>10^6$	4	3	1	0	1	2
Total	17	10	4	1	8	4

vironment or dissemination from nose and throat. As the strains in air and skin samples were identical with the respective perineal strains, contamination from the surroundings must have been insignificant. Apparently, dispersal from the nose and throat were also of minor importance. The results reported in chapter IV showed that individuals whose nasal counts did not exceed those of the 14 perineal carriers, dispersed far fewer staphylococci; and that throat carriers dispersed even less. In addition, 5 patients had different strains in nose and throat from those on the perineum, the former not being demonstrated in skin and air samples. The 14 perineal carriers did not have other staphylococcal multiplication sites of any significance (lesions, axillae, vagina, faeces). Apparently, most of the organisms on the upper lip, fingers, hands, area round the perineum and in the air samples were due to dispersal from the perineum.

The fact that some perineal carriers are heavy dispersers and others light, must, according to the present results, mainly be due to the number of staphylococci on the perineum even though individual variations are quite large. The results of the examinations of patients 9 and 10 support this view. These patients had few perineal staphylococci on the first examination but on the second examination, some days later, the numbers had risen considerably and dispersal to other skin areas and into the air was also markedly greater than before.

In contrast, Ridley (107) found little correlation between the numbers of staphylococci on the perineum and in samples of skin and air contamination. However, he did not examine his patients under standardized conditions and his technique allowed only a crude classification of the bacterial counts. The large individual variations may, therefore, have masked a possible correlation. On the other hand, Ridley stated that there was usually good correlation between contamination of the fingers and clothes and the degree of dispersal into the air.

The perineal carriers had fewer staphylococci on the upper lip, fingers and hands than the nasal carriers (chapter IV), but they were capable of dispersing much larger numbers into the air. Patients 13 and 14, for example, dispersed as many staphylococci into the air as all the 100 nasal carriers in chapter IV together. Apart from contamination via the hands, direct transfer of staphylococci probably takes place from the perineum and surrounding skin to the bedclothes. This pronounced capacity of perineal carriers to disperse the organisms into the air shows that the crux of the staphylococcal problem may not lie solely in nasal carriage. This is probably one of the reasons why treatment of nasal carriers with antibiotic nasal spray has failed to reduce the post-operative infection rate.

Treatment of perineal carriers with hexachlorophane skin disinfection very effectively reduced the number of staphylococci on the skin and dispersal into the air. This is in accordance with the considerable reduction of the skin flora following the use of hexachlorophane liquid cream (9, 49, 83, 85, 118). In maternity departments, washing infants with hexachlorophane emulsion has also considerably reduced the frequency of nasal carriers and staphylococcal lesions (64, 101). Hexachlorophane emulsion is therefore a valuable measure for reducing the dispersal of staphylococci from perineal carriers.

4. *Summary and conclusions.*

1. Staphylococcal skin counts and aerial dispersal on bed making were investigated in 14 patients who were mainly perineal carriers.

2. The numbers of staphylococci on the upper lip, fingers, hands and the region around the perineum increased with rising perineal counts. This also applied to aerial dissemination of staphylococci on bed making.

3. The heaviest dispersers among perineal carriers dispersed much larger numbers of staphylococci into the air than nasal carriers (chapter IV). Perineal carriers may therefore be a greater problem in the control of hospital infection than their frequency would suggest.

4. Five perineal carriers were treated by washing the perineum and adjacent areas with hexachlorophane emulsion. The number of staphylococci on the skin and the aerial dissemination fell from high pre-treatment values to practically nil after completing treatment.

Side-effects of treatment were not observed.

Washing with hexachlorophane emulsion is therefore regarded as a valuable measure for controlling the dispersal of staphylococci from perineal carriers.

VII. The effect of hexachlorophane treatment on self-contamination and dispersal of Staph. aureus by patients with staphylococcal dermal lesions.

A. Previous investigations.

Patients with staphylococcal lesions are regarded as "dangerous carriers". The lesions are often caused by strains resistant to several antibiotics and presumably more virulent than sensitive organisms (7). Further, it has been shown that individuals with staphylococcal lesions have probably been the source of infections in maternity units (5, 34), and in medical and surgical wards (6, 94, 124).

Patients with widespread, staphylococcal-infected skin lesions are among the heaviest dispersers (26, 56). Thomas and Griffiths (125) found that the air of wards housing patients with skin diseases, contained considerably greater numbers of staphylococci than the air of other wards.

We know that patients with staphylococcal-infected skin lesions can disperse large numbers of Staph. aureus, but little is known as to how effectively this dispersal can be reduced. In the present investigation, the skin contamination and aerial dispersal rate of patients with staphylococcal dermal lesions were determined before and after hexachlorophane treatment.

B. Personal investigations.

1. *Material and methods.*

The material consists of 9 women and 6 men between the ages of 17 and 83 years. Two patients (Nos. 1 and 2) had pyodermias involving both thighs, buttocks and the lower part of the back and abdomen. The lesions covered about 30—40 per cent of the total skin surface. Five patients (Nos. 3, 4, 5, 6 and 7) had small infected eczemas in the perineal, inguinal and pubic regions, four (Nos. 8, 9, 10 and 11) had small sores on the hands and one (No. 10) also on the right leg. One patient (No. 12) was examined on two separate admissions to hospital. On the first occasion, she had furuncular residua on the back, and on the second, considerable numbers of staphylococci were demonstrated in the left axilla, although there was no visible lesion. The remaining three patients had various minor skin lesions, such as infected eczemas in both auditory meatus (No. 13), hidrosadenitis in both axillae (No. 14) and infected atheroma on the left leg (No. 15).

All patients were examined once daily

for 2—3 consecutive days. In 14 of the 15 patients, the lesions were washed morning and evening with 3 per cent hexachlorophane emulsion (pHaisoHex) and the patients were re-examined during and after treatment.

After preliminary instruction the patients carried out the treatment themselves. A procedure similar to that described in chapter VI was employed. Those with perineal staphylococci treated the perineum as well, the rest of the body being washed with ordinary, non-disinfectant soap. Three patients (Nos. 1, 2 and 12) were unable to manage the treatment themselves and were therefore washed by ward nurses. The patient with auditory meatal eczemas was treated with tampons moistened in a solution of 10 ml hexachlorophane emulsion in 90 ml water. Four to 30 ml hexachlorophane emulsion were used at each treatment, depending on the size of the skin lesion.

Two patients (Nos. 4 and 11) were also treated daily with framycetin-gramicidin nasal spray and one patient (No. 2) with fucidic acid 0.5 g × 3. Apart from these instances, chemotherapeutic or skin disinfecting agents other than hexachlorophane emulsion were not used.

To achieve approximately equal experimental conditions before, during and after treatment, the patients were bathed or washed on a stretcher and received clean clothes and bedclothes 2 days before the first pre-treatment examination and again 2 days before each examination during and after treatment.

The investigation methods are described in chapter II. Three patients (Nos. 1, 2 and 12) had such extensive skin lesions that only a 25 sq. cm area was examined. From the remaining 12 patients, the samples were obtained from the whole affected skin area. The sampling fluids and media used during and after treatment contained 1 per cent "Tween 80" in order to neutralize possible residues of hexachlorophane emulsion.

2. *Results.*

Quantitative estimations.

Table 45 gives the results of the examinations before, during and after treatment. Before treatment the dispersal of staphylococci into the air from the 2 patients with extensive pyodermias was about 3 times as great as for all the other patients together. Calculated on the basis of the mean counts, patient 1 had more staphylococci on the fingers and hands than all the other patients together. The 4 patients with infected hand sores yielded large counts from the hands, but air contamination was somewhat less than for the patients with infected eczemas in the pubic, perineal and inguinal regions. Considerable numbers of staphylococci were isolated from the lesions in patients 13, 14 and 15 but air contamination was moderate.

Fourteen patients were treated by washing with hexachlorophane. In all these, there was a marked reduction in staphylococcal counts, from high pre-treatment values to very modest numbers during the treatment and after its completion. To illustrate this relationship three patients will be discussed in detail.

Fig. 18 gives the results of treatment of patient 1. Before therapy, the patient constantly developed yellow-green bullae which burst and dried into crusted sores up to 5 × 5 cm. After treatment was instituted this process ceased and 4 days later the skin and air contamination were considerably reduced. However, the number of staphylococci in these samples did not fall to modest levels until 11 days after the commencement of treatment. Therapy was

Table 45. *Skin contamination and aerial dissemination of staphylococci in relation to treatment (hexachlorophane emulsion); 15 patients with staphylococcal dermal lesions. No. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	No. of days treated	Day of treat-ment	Dermal lesion	Nose	Throat	Upper lip	Fingers	Hands	Peri-neum	Air conta-mination (bed making)
1 F 74	25	÷ 3	2,080.00	408.00	2.40	2.60	1,610.00	8,056.0	2,800.00	1,049.960
		÷ 2	6,480.00	488.00	2.80	0.76	1,140.00	6,240.0	4,440.00	1,353.240
		÷ 1	2,960.00	1,240.00	2.00	0.28	710.00	1,080.0	1,880.00	997.500
		4	320.00	1,872.00	3.20	3.24	16.00	120.0	240.00	99.120
		8	8.00	618.00	7.20	0.34	2.42	24.0	<0.08	15.120
		11	<0.08	602.00	8.80	0.12	0.48	1.5	<0.08	0.840
2 F 20	9	÷ 2	280.00	236.00	1.56	2.80	100.00	842.0	2,840.00	1,113.420
		÷ 1	810.00	288.00	1.32	3.64	92.00	680.0	9,680.00	1,170.120
		3	<0.08	256.00	0.08	0.08	5.00	28.0	80.00	15.540
		5	<0.08	320.00	0.96	0.04	0.08	<0.5	16.00	0.840
		7	<0.08	<0.04	<0.04	<0.02	0.04	<0.5	0.40	0.420
		9	<0.08	<0.04	<0.04	<0.02	<0.02	<0.5	<0.08	<0.420
		+ 8	<0.08	0.24	<0.04	<0.02	<0.02	<0.5	<0.08	<0.420
		+17	<0.08	316.00	0.92	0.44	0.30	21.0		3.700
3 F 61	4	÷ 2	1,840.00	183.00	0.12	0.02	3.48	80.0	480.00	72.800
		÷ 1	840.00	641.00	0.52	0.66	8.44	112.0	368.00	70.400
		2	5.52	1,368.00	0.24	0.08	0.20	<0.5	<0.08	0.700
		4	<0.08	248.00	2.16	<0.02	<0.02	<0.5	<0.08	0.100
4 M 17	5	÷ 3	5,240.00	368.00	<0.04	0.06	4.00	36.0	304.00	145.400
		÷ 2	964.00	524.00	<0.04	0.42	33.20	130.0	355.00	132.400
		÷ 1	3,120.00	0.60	<0.04	0.24	60.00	138.0	836.00	200.900
		2	4.00	0.08	0.12	<0.02	0.12	1.0	<0.08	3.500
		4	0.80	<0.04	0.34	<0.02	0.12	<0.5	<0.08	0.300
		6	0.08	<0.04	0.18	<0.02	<0.02	<0.5	<0.08	<0.025
5 M 29	9	÷ 3	680.00	224.00	0.24	3.40	63.00	40.0	96.00	17.300
		÷ 1	320.00	400.00	7.28	0.44	25.00	26.0	72.00	21.400
		4	<0.08	92.00	8.00	<0.02	<0.02	<0.5	<0.08	<0.025
6 M 17	2	÷ 2	4,120.00	920.00	0.12	0.08	4.00	20.0	360.00	54.000
		÷ 1	1,040.00	280.00	0.24	<0.02	4.08	210.0	92.00	32.000
		2	5.60	1,400.00	<0.04	<0.02	0.16	1.5	4.00	2.000
7 M 23	18	÷ 3	8,240.00	60.00	0.04	9.00	5.32	60.0		29.900
		÷ 2	4,840.00	160.00	0.16	1.00	10.56	140.0		37.400
		÷ 1	2,440.00	40.00	<0.04	0.16	2.18	20.0		38.600
		4	16.00	160.00	<0.04	0.02	0.08	<0.5		0.300

÷ = before treatment. + = after treatment.

Table 45 cont. *Skin contamination and aerial dissemination of staphylococci in relation to treatment (hexachlorophane emulsion); 15 patients with staphylococcal dermal lesions. No. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	No. of days treated	Day of treat-ment	Dermal lesion	Nose	Throat	Upper lip	Fingers	Hands	Peri-neum	Air conta-mination (bed making)
8 F 18	3	÷ 3	320.00	120.00	0.32	0.16	7.86	1,470.0	<1.00	8.900
		÷ 2		668.00	0.64	0.92	0.24	1,620.0	<1.00	5.500
		÷ 1		40.00	8.00	0.44	11.84	1,040.0	<1.00	4.900
		3	<0.04	112.00	208.00	<0.02	<0.02	<0.5	<1.00	0.200
		+ 1		332.00	4.80	1.52	0.02	0.5	<1.00	0.050
9 M 42	5	÷ 3	840.00	324.00	<0.04	0.04	1.44	1,200.0	<1.00	13.200
		÷ 2		228.00	<0.04	1.96	2.48	2,080.0	<1.00	17.000
		÷ 1	320.00	344.00	<0.04	0.08	2.64	880.0	<1.00	10.900
		4	<0.04	860.00	<0.04	<0.02	0.02	<0.5	<1.00	0.100
10 F 22	5	÷ 2	*2.00	3.00	<0.04	0.02	93.00	320.0	<1.00	2.600
		÷ 1	1.64	4.00	<0.04	0.02	41.00	160.0	<1.00	3.500
		3	<0.02	8.00	<0.04	0.04	<0.02	<0.5	<1.00	0.025
11 F 55	4	÷ 6		2,920.00	0.08	0.72	0.56	172.0	<1.00	3.500
		÷ 1	102.00	<0.04	0.04	<0.02	0.80	236.0	<1.00	2.500
		2	<0.04	<0.04	<0.04	<0.02	<0.02	<0.5	<1.00	<0.025
		+ 2	<0.04	<0.04	<0.04	<0.02	<0.02	<0.5	<1.00	<0.025
12 F 24	3	÷ 4	0.84	152.00	0.08	0.06	0.30	<0.5	<1.00	13.300
		÷ 3	0.30	168.00	<0.04	0.46	0.22	7.0	<1.00	10.200
		÷ 1	0.36	64.00	<0.04	0.02	0.40	<0.5	<1.00	10.000
		3	<0.04	60.00	0.08	<0.02	0.12	<0.5	<1.00	<0.025
	—	÷ 3	8.40	200.00	<0.04	1.12	0.20	5.0	<1.00	0.700
		÷ 2	16.80	280.00	<0.04	<0.02	<0.02	1.0	<1.00	1.300
		÷ 1	52.80	80.00	<0.04	0.12	0.36	4.0	<1.00	0.700
13 F 72	3	÷ 2	10,400.00	20.00	<0.04	0.04	1.92	140.0	<1.00	4.800
		÷ 1	20,400.00	20.00	0.08	<0.02	3.46	160.0	<1.00	3.900
		3	<0.04	188.00	<0.04	<0.02	0.08	<0.5	<1.00	0.100
14 F 65	—	÷ 2	46,400.00	8.00	0.12	<0.02	0.32	6.5	<1.00	2.500
		÷ 1	120800.00	8.00	<0.04	<0.02	0.12	12.5	<1.00	2.000
15 M 56	6	÷ 3	11,200.00	1.00	<0.04	2.24	1.60	340.0	<1.00	3.300
		÷ 1	4,760.00	0.40	<0.04	2.80	0.24	80.0	<1.00	7.200
		5	<0.04	0.08	0.44	<0.02	<0.02	<0.5	<1.00	<0.025

* From lesion on right leg.

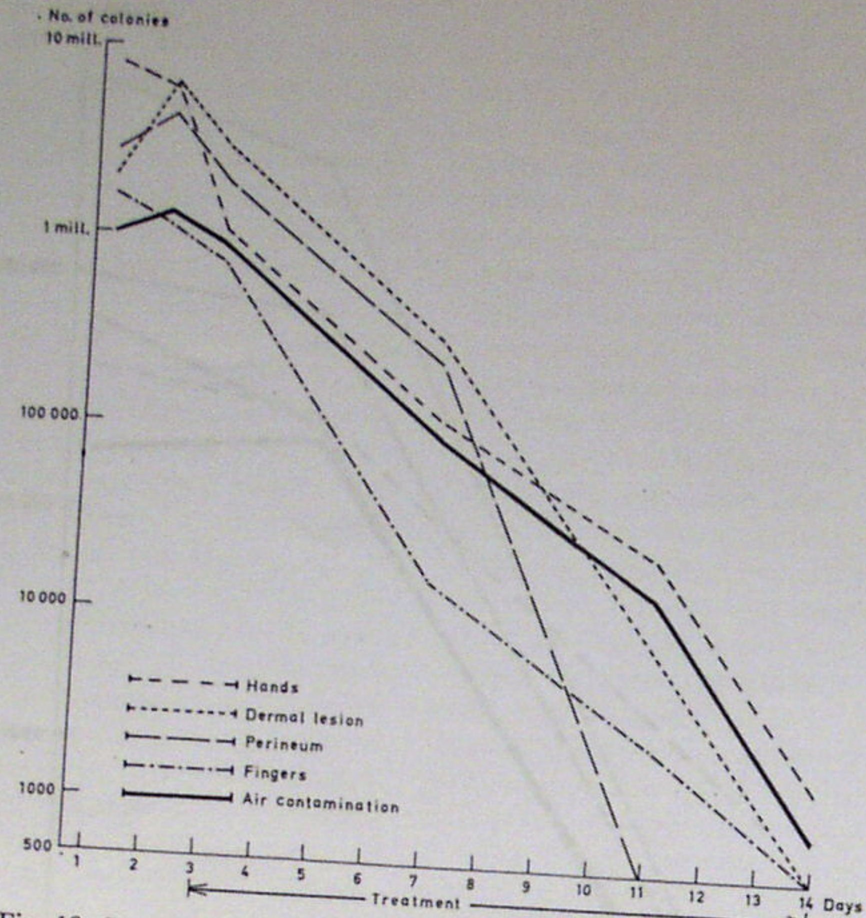

Fig. 18. Staphylococcal counts from patient 1 before, during and after treatment with hexachlorophane emulsion.

continued and the patient was discharged 14 days later. The affected skin area was healed. The nasal and throat counts were about the same as before treatment.

Fig. 19 gives the results of treatment of patient 2. After 3 days' therapy skin and air contamination were considerably reduced and 2 days later only small numbers of staphylococci were demonstrated in these samples. From then on the patient also received fucidic acid therapy for 4 days. During this treatment, the staphylococci in nasal and throat cultures also disappeared and on the 9th day of treatment all samples were negative and the skin lesion was almost healed. Therapy was then stopped. After 8 and 17 days rising counts were demonstrated in nasal cultures, and skin and air samples yielded a few organisms. The skin lesion had healed.

Fig. 20 gives the results of treatment of patient 5, who had acute hepatitis. He sweated profusely and suffered intense pruritus. Seven days after admission, he developed a staphylococcal-infected eczema in the pubic region. Identical strains were isolated from the eczema as had been isolated from the nose and perineum at the time of admission. The patient had a different strain in his throat. He was a marked staphylococcal disperser but after

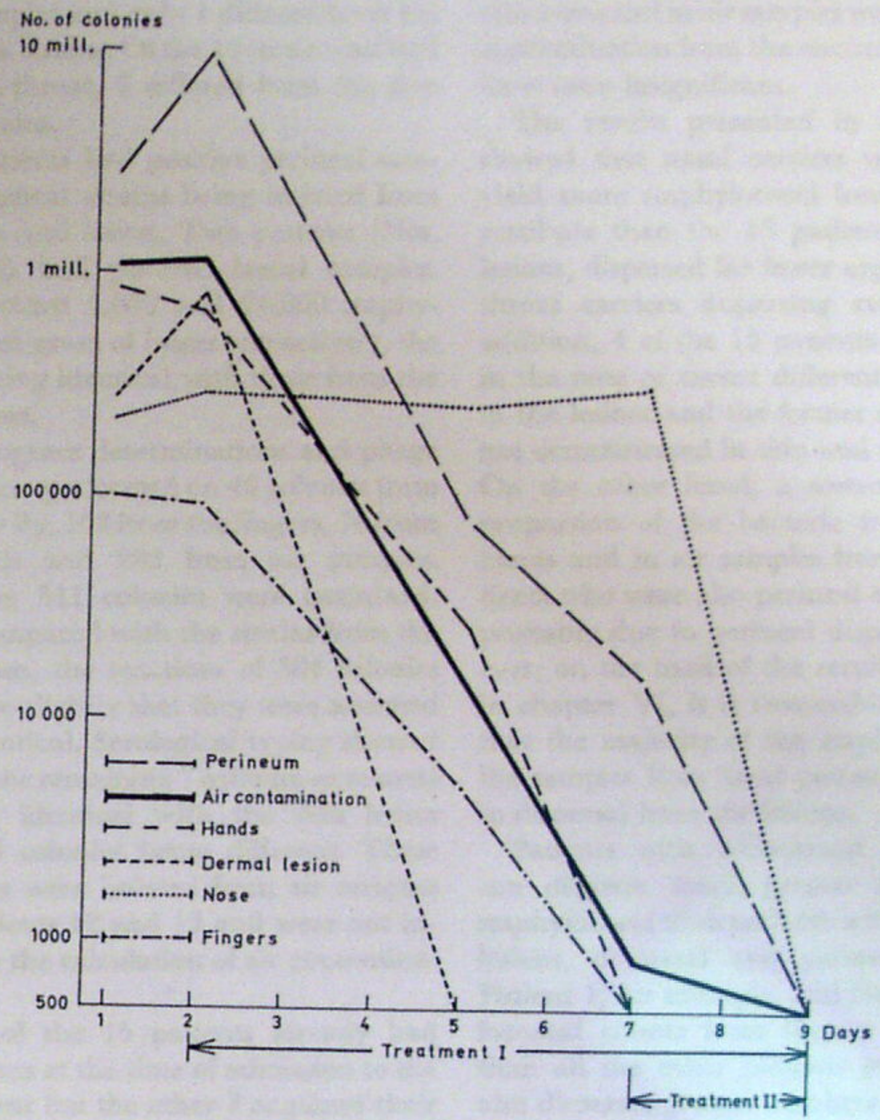

Fig. 19. Staphylococcal counts from patient 2 before, during and after treatment with hexachlorophane emulsion (I) and fucidic acid (II).

2 days' treatment with hexachlorophane, no staphylococci were demonstrated in skin and air samples.

For the remaining patients, a reduction in the staphylococcal counts to minimal values was observed after only 2—5 days' treatment. The skin lesions had begun to heal. Patients with pruritus of the affected skin areas noticed a considerable improvement after only 1—2 days' treatment. Therapy was continued until the lesions were healed; this took 6 to 25 days.

Patients 4 and 11 were treated with framycetin-gramicidin nasal spray 4 times daily for 1 and 4 days respectively, before they received hexachlorophane treatment. After spray therapy, the nasal counts fell practically to nil, while the skin and air samples yielded about the same number as before treatment.

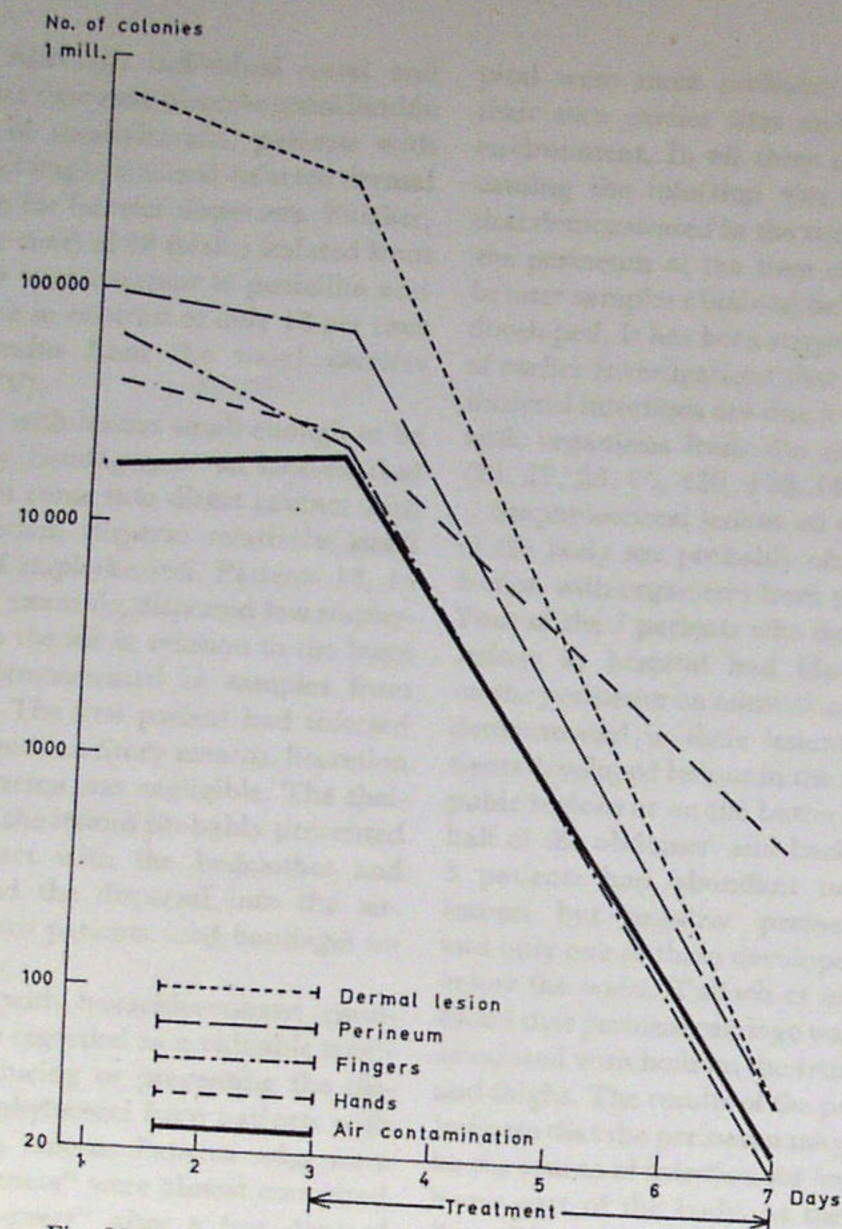

Fig. 20. Staphylococcal counts from patient 5 before and after treatment with hexachlorophane emulsion.

Antibiogram and phage patterns.

Antibiogram determinations and phage typing were performed on 71 staphylococcal colonies from the lesions. Altogether 18 strains were demonstrated. Two patients had 2 and 3 strains respectively. Ten strains (56 per cent) were resistant to penicillin and tetracycline.

Sixteen strains were lysed by the phages used. Five strains belonged to phage group III, 4 strains to phage group I and 4 strains to the miscellaneous group. There were 1 and 2 strains respectively in phage groups II and V.

All patients had positive nasal cultures and 13 had positive throat cultures. Eighteen strains were demonstrated in the

nasal samples and only 1 differed from the skin lesion strains. Of the 14 strains isolated from the throat, 3 differed from the skin lesion strains.

Six patients had positive perineal samples, identical strains being isolated from perineum and lesion. Two patients (Nos. 1 and 4) had positive faecal samples. These yielded 5,000 and 24,000 staphylococci per gram of faeces respectively, the strains being identical with those from the skin lesions.

Antibiogram determinations and phage typing were performed on 46 colonies from the upper lip, 108 from the fingers, 74 from the hands and 283 from air samples. Altogether 511 colonies were examined. When compared with the strains from the skin lesions, the reactions of 504 colonies differed so slightly that they were assumed to be identical. Serological typing showed that 4 of the remaining 7 colonies were most probably identical with the skin lesion strains, 3 colonies being different. These 3 colonies were isolated from air samples from patients 12 and 13 and were not included in the calculation of air contamination.

Eight of the 15 patients already had their lesions at the time of admission to the department but the other 7 acquired their lesions after admission. In these 7 patients, the skin lesion strains were identical with those isolated from nose, throat or perineum before the lesions became manifest.

3. *Discussion.*

An essential problem in this study was whether the staphylococci on the upper lip, fingers and hands and in the air samples really were due to dispersal from the skin lesions and not to contamination from the environment or dispersal from nose, throat or perineum. As the strains from the lesions and those from the other skin areas and in air samples were identical, contamination from the environment must have been insignificant.

The results presented in chapter IV showed that nasal carriers who did not yield more staphylococci from the nasal vestibule than the 15 patients with skin lesions, dispersed far fewer organisms, the throat carriers dispersing even less. In addition, 4 of the 15 patients had strains in the nose or throat different from those in the lesions and the former strains were not demonstrated in skin and air samples. On the other hand, a somewhat larger proportion of the bacteria from fingers, hands and in air samples from the 6 patients who were also perineal carriers, was probably due to perineal dispersal. However, on the basis of the results presented in chapter VI, it is reasonable to assume that the majority of the staphylococci in the samples from these patients were due to dispersal from the lesions.

Patients with widespread pyodermias can disperse much greater numbers of staphylococci than patients with small skin lesions, or nasal and perineal carriers. Patient 1, for example, had larger staphylococcal counts from fingers and hands than all the other patients together and also dispersed greater numbers into the air. The 100 nasal carriers previously mentioned (chapter IV), taken together, had about the same number of staphylococci on the fingers and hands as patient 1. The aggregate count from these skin areas of the 14 perineal carriers mentioned earlier (chapter VI) was lower than that of patient 1. Patients 1 and 2 dispersed more staphylococci into the air than the other patients with lesions, the 14 perineal carriers and the 100 nasal carriers altogether. Air samples from patient 1 yielded about 500 times as many colonies as the mean value for air samples from the 100 nasal

carriers. Although individual nasal and perineal carriers may disperse considerable numbers of staphylococci, patients with widespread staphylococcal-infected dermal lesions are far heavier dispersers. Further, 10 (56 per cent) of 18 strains isolated from the lesions were resistant to penicillin and tetracycline in contrast to only 18 per cent of the strains from the nasal carriers (chapter IV).

Patients with lesions small enough to be covered by bandages, or so located that they do not come into direct contact with the bedclothes, disperse relatively small numbers of staphylococci. Patients 13, 14 and 15, for example, dispersed few staphylococci into the air in relation to the large numbers demonstrated in samples from the lesions. The first patient had infected eczema of both auditory meatus. Secretion from the eczema was negligible. The sheltered site of the lesions probably prevented direct contact with the bedclothes and thus reduced the dispersal into the air. The other two patients used bandages on their lesions.

Washing with hexachlorophane emulsion must be regarded as a valuable treatment for reducing or preventing the dispersal of staphylococci from patients with infected skin lesions. Patients who were "heavy dispersers" were almost converted to "non-dispersers" after a few days of this treatment alone, and their skin lesions began to heal. Patients who were also nasal carriers had remarkably few staphylococci on fingers and hands during treatment though they still had large numbers in nasal cultures. Hexachlorophane therapy probably also reduces the contamination of the hands with nasal staphylococci.

Autoinfection probably plays an important part in the epidemiology of staphylococcal infections. All the 7 patients who acquired their infections while in hospital were most probably infected from their own carrier sites and not from the environment. In all these cases the strain causing the infection was identical with that demonstrated in the nose, throat or on the perineum at the time of admission or in later samples obtained before the lesions developed. It has been suggested in a series of earlier investigations that many staphylococcal infections are due to autoinfection with organisms from the nasal vestibule (23, 27, 28, 65, 130, 140, 148).

Staphylococcal lesions on the lower half of the body are probably often due to infection with organisms from the perineum. Four of the 7 patients who developed their lesions in hospital had identical strains on the perineum on admission as were later demonstrated in their lesions. These patients developed lesions in the inguinal and pubic regions or on the buttocks and lower half of the abdomen and back. The other 3 patients had abundant nasal staphylococci but negative perineal samples, and only one of them developed infections below the waist. Tulloch et al. (127) also found that perineal carriage was sometimes associated with boils on the trunk, buttocks and thighs. The results of the present study indicate that the perineum may quite often be the source of infection for lesions on the lower part of the body, as mentioned by Kay (74), and that the rest of the body is most frequently infected from the nose.

4. *Summary and conclusions.*

1. The numbers of Staph. aureus on various skin sites and the ability to disperse the organisms into the air on bed making were investigated in 15 patients with staphylococcal skin lesions. Two patients had extensive pyodermias. One patient had bilateral axillary hidrosadenitis and the other had small infected eczemas, sores, or furuncular residua.

2. Greater numbers of staphylococci were dispersed into the air from each of the patients with pyodermias than from the other 13 patients together. One of the pyodermia patients also yielded more staphylococci on the fingers and hands than the other 14 patients together.

Patients with widespread staphylococcal-infected skin lesions are "heavy dispersers" of staphylococci. As their strains are often resistant to several antibiotics they should also be regarded as "dangerous carriers".

3. Fourteen patients were treated by washing their skin lesions with hexachlorophane emulsion. In the course of 2—11 days, all were converted from "heavy dispersers" almost to "non-dispersers" and at the same time, the lesions began to heal.

No side-effects of treatment were observed.

Washing with hexachlorophane emulsion is regarded as a valuable measure for preventing staphylococcal dispersal from patients with staphylococcal skin lesions.

4. Seven of the 15 patients developed their lesions while in hospital. In all these patients, the strains causing the lesions were identical with those demonstrated in the nose, throat or on the perineum before the lesions developed. It is assumed that the patients were infected with organisms from their own carrier sites and that the perineum may quite often be the source of infections on the lower part of the body.

VIII. General summary and conclusions.

I. The purpose of the present investigation was primarily to study why staphylococcal carriers differ in their ability to disperse their organisms into the air and secondly to investigate the effect of antibiotic nasal spray and hexachlorophane skin disinfection in reducing the staphylococcal dispersal from individual carriers.

II. To study these problems suitable methods of quantitative estimations of staphylococci on different sites of the body were evaluated. To assess the ability of individuals to disperse staphylococci into the air a standardized form of bed making in a test room was developed.

The capacity to coagulate rabbit plasma was used as the only criterion for the selection of pathogenic staphylococci. Antibiogram determinations, phage typing and serological typing were used to determine whether staphylococcal strains isolated from different sites of an individual were identical.

III. The design of experiments is described.

Staphylococcal carriers among the patients admitted to Medical Department B, Haukeland Hospital, Bergen were examined.

IV. One hundred persistent nasal carriers of Staph. aureus were selected.

Staphylococcal counts from upper lip, fingers and hands increased with increasing nasal counts. In every case the nasal and skin strains were identical.

The majority of Staph. aureus on the skin of nasal carriers are derived from the nasal vestibule.

The dispersal of Staph. aureus into the air on bed making also increased with increasing nasal counts but there was better correlation between skin (fingers and hands) and air counts than between nasal and air counts. Ninety-eight per cent of the strains in air samples were identical with the nasal and skin strains.

The heaviest dispersers of staphylococci among nasal carriers are those who yield the highest numbers of organisms on the skin (fingers and hands). Usually they also have the highest nasal counts.

Throat-carriers disperse far less organisms than nasal carriers.

V. Two groups of 20 and 30 persistent nasal carriers of Staph. aureus were treated with framycetin-gramicidin nasal spray 4 times daily for 3 and 7 days respectively.

The staphylococcal nasal counts fell from high pre-treatment values to less than 0.01 per cent of the original counts on the day after completion of treatment. They remained relatively low for 4—7 days, then rose during the following week to about the same level as before treatment.

Forty persistent nasal carriers were treated with framycetin-gramicidin nasal spray for 3 days. The counts from nose, upper lip, fingers and hands fell from high pre-treatment values almost to nil the day after completion of therapy. The same applied to aerial dissemination on bed making.

When the nasal counts increased after treatment, the skin and air counts also increased.

Framycetin-gramicidin nasal spray is a valuable measure for preventing staphylococcal dissemination from nasal carriers.

VI. The numbers of staphylococci on various skin areas of 14 persistent perineal carriers and the degree of aerial dissemination on bed making increased with increasing perineal counts.

The heaviest dispersers among the perineal carriers dispersed far greater numbers of staphylococci into the air than the nasal carriers (chapter IV). Perineal carriers represent a greater problem in the control of hospital infection than their frequency would suggest.

Five perineal carriers were treated by washing their perineum and adjacent areas with hexachlorophane emulsion. The skin and air counts fell from high pre-treatment values practically to nil. Hexachlorophane skin disinfection is a valuable measure for controlling the staphylococcal dispersal from perineal carriers.

VII. Self-contamination and aerial dispersal of Staph. aureus were investigated in 15 patients with various staphylococcal skin lesions.

Two patients with extensive pyodermias yielded far greater skin and air counts than individuals with minor skin infections. Patients with widespread staphylococcal-infected skin lesions are among the heaviest dispersers.

Fourteen patients were treated by washing their skin lesions with hexachlorophane emulsion. In the course of 2—11 days, all were converted from heavy dispersers almost to non-dispersers, and the lesions began to heal. Hexachlorophane skin disinfection is a valuable measure for preventing dispersal of Staph. aureus from patients with staphylococcal skin lesions.

Seven patients developed their lesions while in hospital. The strains causing the lesions were identical with those demonstrated in nose, throat or on the perineum before the lesions developed.

References

1. ABRAHMSON, B. P. and SMORODINZEFF, A. A.: Experimentelle Untersuchungen zur Frage der mechanischen Händedesinfektion. Zbl. Chir., II: 2125–2130, 1928.
2. ANDERSON, E. S. and WILLIAMS, R. E. O.: Bacteriophage typing of enteric pathogenes and staphylococci and its use in epidemiology. J. clin. Path., 9: 94–127, 1956.
3. BAILEY, J. and FOSTER, D. W.: "Phiso-Hex and the food handler". The Sanitarian, (London), 69: 250–256, 1961.
4. BAKER, W. H. J. and CHRISTIE, D. R.: Breast absess. Brit. med. J., II: 192, 1959.
5. BARBER, M. and BURSTON, J.: Antibiotic-resistant staphylococcal infection. A study of antibiotic sensitivity in relation to bacteriophage types. Lancet, II: 578–582, 1955.
6. BARBER, M. and DUTTON, A. A. C.: Antibiotic-resistant staphylococcal outbreaks in a medical and a surgical ward. Lancet, II: 64–68, 1958.
7. BARBER, M., DUTTON, A. A. C., BEARD, M. A., ELMES, P. C. and WILLIAMS, R.: Reversal of antibiotic resistance in hospital staphylococcal infection. Brit. med. J., I: 11–17, 1960.
8. Barber, M. and Warren, S.: Control of cross-infection in a surgical ward. Lancet, II: 374–378, 1962.
9. BEILBY, J. O. W. and THOMPSON, R. E. M.: Antisepsis of surgeons' hands. A study of various agents under theatre conditions. Brit. J. Surg., 48: 598–600, 1961.
10. BIE, K.: Resistant staphylococci and airborne bacteria in a surgical department. J. Oslo Cy Hosp., 9: 132–178, 1959.
11. BLAIR, J. E. and WILLIAMS, R. E. O.: Phage typing of staphylococci. Bull. Wld Hlth Org., 24: 771–784, 1961.
12. BLOWERS, R. and WALLACE, K. R.: The sterilisation of blankets with cetyl trimethylamine bromide. Lancet, I: 1250–1251, 1955.
13. Bowers, A. G.: Germicidal liquid. Soap, 26 (No. 8): 36–37, 1950.
14. BRODIE, J., KERR, M. R. and SOMMERVILLE, T.: The hospital staphylococcus. A comparison of nasal and fæcal carrier states. Lancet, I: 19–20, 1956.
15. BRUINING, M. and COHEN, H. H.: An investigation about "anticoagulase", a ferment said to be produced by staphylococci. Antonie v. Leeuwenhoek, 14: 87–96, 1948.
16. Burtenshaw, J. M. L.: The mortality of the haemolytic streptococcus on the skin and on other surfaces. J. Hyg., Camb., 38: 575–586, 1938.
17. BUTTIAUX, R. and PIERRET, J.: Les staphylocoques pathogènes dans les selles des nourrissons normaux. Ann. Inst. Past., 76: 480–482, 1949.
18. BØE, J. and SOLBERG, C. O.: Bruk av bonemaskiner på sykehus. T. norske Lægeforen., 83: 341–344, 1963.
19. BØE, J., SOLBERG, C. O., VOGELSANG, Th. M. and WORMNES, A.: Perineal carriers of staphylococci. Brit. med. J., II: 280–281, 1964.
20. CADE, A. R.: Antiseptic soaps. A simplified in-vivo method for determining their degerming efficiency. Soap, 26 (No. 7): 35–38, 1950.
21. CHAPMAN, G. H.: The significance of sodium chloride in studies of staphylococci. J. Bact., 50: 201–203, 1945.
22. CHAPMAN, G. H.: A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation and the Stone reaction. J. Bact., 51: 409–410, 1946.
23. COLBECK, J. C., ROCKE ROBERTSON, H., SUTHERLAND, W. H. and HARTLEY, F. C.: The importance of endogenous staphylococcal infections in surgical patients. Canad. Serv. med. J., 15: 326–330, 1959.
24. COLEBROOK, L.: Memorandum on the sterilisation of the hands. Ministry of health. Appendix to interim report of departemental committee on maternal mortality and morbidity. London, 1930, pp. 122–135.
25. COMTOIS, P. O. and BYNOE, E. T.: Lytic reactions of three closely related staphylococcal bacteriophages 80, 81 and "52AV". Bacteriol. Proc., p. 139, 1958.
26. COOKE, E. M. and BUCK, H. W.: Self-contamination of dermatological patients with Staphylococcus aureus. Brit. J. Derm., 75: 21–25, 1963.
27. Copeman, P. W. M.: Treatment of recurrent styes. Lancet, II: 728–729, 1958.
28. DANBOLT, N.: Undersøkelser over stafylokokker med særlig henblikk på furunkulosens epidemiologi. Skr. norske Vidensk.-Akad., I. Mat.-nat. Kl. 1931. No. 10.
29. DAVIES, R. R. and NOBLE, W. C.: Dispersal of bacteria on desquamated skin. Lancet, II: 1295–1297, 1962.
30. DEVENISH, E. A. and MILES, A. A.: Control of Staphylococcus aureus in an operating-theatre. Lancet, I: 1088–1094, 1939.
31. DUGUID, J. P. and WALLACE, A. T.: Air infection with dust liberated from clothing. Lancet, II: 845–849, 1948.
32. EICHENWALD, H. F., KOTSEVALOV, O. and FASSO, L. A.: Another example of bacterial viral interaction: "The cloud baby", an important factor in the transmission of staphylococcal infection in the nursery and in the home. Amer. J. Dis. Child., 98: 432, 1959.
33. ELEK, S. D. and FLEMING, P. C.: A new technique for the control of hospital cross-infection. Experience with BRL. 1241 in a maternity unit. Lancet, II: 569–572, 1960.
34. ELLIOTT, S. D., GILLESPIE, E. H. and HOLLAND, E.: An outbreak of "pemphigus neonatorum" in a maternity home. Lancet, I: 169–171, 1941.
35. Enticott, B. G. H.: Some aspects of staphylococcal skin infections. N.Z. med. J., 55: 237–242, 1956.
36. ERICSSON, H.: Rational use of antibiotics in hospitals. Studies on laboratory methods and discussion of the biological basis for their clinical application. Scand. J. clin. Lab. Invest., 12: Suppl. 50, 1960.
37. ERIKSEN, K. RIEWERTS and LUND, F.: Undersøgelser over et kvarternært ammoniumklorids (Rodalon) huddesinficerende egenskaber. Ugeskr. Læg., 115: 336–341, 1953.
38. FAGRAEUS, A.: Influence of sodium chloride on growth of staphylococci and some other bacteria. Acta path. microbiol. scand., 26: 655–665, 1949.
39. GILLESPIE, E. H., DEVENISH, E. A. and COWAN, S. T.: Pathogenic staphylococci. Their incidence in the nose and on the skin. Lancet, II: 870–873, 1939.
40. GILLESPIE, W. A. and ALDER, V. G.: Control of an outbreak of staphylococcal infection in a hospital. Lancet, I: 632–634, 1957.
41. GILLESPIE, W. A., SIMPSON, K. and TOZER, R. C.: Staphylococcal infection in a maternity hospital. Epidemiology and control. Lancet, II: 1075–1080, 1958.
42. GILLESPIE, W. A., ALDER, V. G., AY-

LIFFE, G. A. J., BRADBEER, J. W. and WYPKEMA, W.: Staphylococcal cross-infection in surgery. Effects of some preventive measures. Lancet, II: 781–784, 1959.

43. GILLESPIE, W. A., ALDER, V. G., AYLIFFE, G. A. J., POWELL, D. E. B. and WYPKEMA, W.: Control of staphylococcal cross-infection in surgical wards. A four-and-a-half-year study. Lancet, I: 1299–1303, 1961.

44. GOULD, J. C. and ALLAN, W. S. A.: Staphylococcus pyogenes cross-infection. Prevention by treatment of carriers. Lancet, II: 988–989, 1954.

45. GOULD, J. C.: The effect of local antibiotic on nasal carriage of Staphylococcus pyogenes. J. Hyg., Camb., 53: 379–385, 1955.

46. GOULD, J. C. and CRUIKSHANK, J. D.: Staphylococcal infections in general practice. Lancet, II: 1157–1161, 1957.

47. GREEN, K. G.: The role of the carrier in staphylococcal disease. Lancet, II: 921–923, 1961.

48. GREMEAUX, A. E.: Treatment of nasal carriers of staphylococci. Lancet, I: 284, 1961.

49. HALVORSEN, J. F. and HOFSTAD, T.: Preoperativ hånddesinfeksjon med pHiso-Hex. Nord. Med., 67: 671–673, 1962.

50. HAMBURGER, M., Jr., GREEN, M. J. and HAMBURGER, V. G.: The problem of the "dangerous carrier" of hemolytic streptococci. I. Number of hemolytic streptococci expelled by carriers with positive and negative nose cultures. J. infect. Dis., 77: 68–81, 1945.

51. HAMBURGER, M., JR., GREEN, M. J. and HAMBURGER, V. G.: The problem of the "dangerous carrier" of hemolytic streptococci. II. Spread of infection by individuals with strongly positive nose cultures who expelled large numbers of hemolytic streptococci. J. infect. Dis., 77: 96–108, 1945.

52. HAMBURGER, M., Jr. and GREEN, M. J.: The problem of the "dangerous carrier" of hemolytic streptococci. IV. Observations upon the role of the hands, of blowing the nose, of sneezing, and of coughing in the dispersal of these microorganisms. J. infect. Dis., 79: 33–44, 1946.

53. Hare, R. and MacKenzie, D. M.: The source and transmission of nasopharyngeal infections due to certain bacteria and viruses. Brit. med. J., I: 865–870, 1946.

54. HARE, R. and THOMAS, C. G. A.: The transmission of Staphylococcus aureus. Brit. med. J., II: 840–844, 1956.

55. HARE, R. and RIDLEY, M.: Further studies on the transmission of Staph. aureus. Brit. med. J., I: 69–73, 1958.

56. HARE, R. and COOKE, E. M.: Self-contamination of patients with staphylococcal infections. Brit. med. J., II: 333–336, 1961.

57. HARE, R.: Dispersal of staphylococci. Pp. 75–86 in infection in hospitals. Epidemiology and control. Ed. Williams, R. E. O. and Shooter, R. A. Blackwell Scientific Publications, Oxford, 1963.

58. HARDYMENT, A. F., WILSON, R. A., COCKCROFT, W. and JOHNSON, B.: Observations on the bacteriology and epidemiology of nursery infections. I. Staphylococcal skin infections. Pediatrics, II: 907–918, 1960.

59. HAUKENES, G. and OEDING, P.: On two new antigens in Staphylococcus aureus. Acta path. microbiol. scand., 49: 237–248, 1960.

60. HAUKENES, G.: Serological typing of Staphylococcus aureus. 1. Factor a serum. Acta path. microbiol. scand., 59: 205–212, 1963.

61. HAUKENES, G.: Serological typing of Staphylococcus aureus. 3. Factor ac and c sera. Acta path. microbiol. scand., 59: 220–228, 1963.

62. HAUKENES, G.: Serological typing of Staphylococcus aureus. 4. Factor h serum. Acta path. microbiol. scand., 60: 285–294, 1964.

63. HENDERSON, R. J. and WILLIAMS, R. E. O.: Nasal disinfection in prevention of post-operative staphylococcal infection of wounds. Brit. med. J., II: 330–333, 1961.

64. HILL, A. M., BUTLER, H. M. and LAVER, J. C.: Reduction of staphylococcal infection in the newly born. Med. J. Aust., 2: 633–634, 1959.

65. HOBBS, B. C., CARRUTHERS, H. L. and GOUGH, J.: Sycosis barbæ. Serological types of Staphylococcus pyogenes in nose and skin and results of penicillin treatment. Lancet, II: 572–574, 1947.

66. HOFSTAD, T. and WORMNES, A.: The effect of broad spectrum antibiotics on the faecal staphylococcal and monilial flora in man. Acta path. microbiol. scand., 51: 275–279, 1961.

67. HOFSTAD, T.: Studies on the antigenic structure of the 80/81 complex of Staphylococcus aureus. 1. Agglutinogens. Acta path. microbiol. scand., 61: 558–570, 1964.

68. HOWE, C. W.: Postoperative wound infections due to Staphylococcus aureus. New Engl. J. Med., 251: 411–416, 1954.

69. HUFNAGEL, C. A., WALTER, C. W. and HOWARD, R. W.: An in vivo method for evaluation of detergents and germicides. Surgery, 23: 753–759, 1948.

70. HÆGLER, C. S.: Händereinigung, Händedesinfektion und Händeschutz. Eine experimentelle und kritische Studie. Basel, Benno Schwabe, 1900.

71. JARVIS, A. W. and WIGLEY, R. D.: Recurrence of staphylococci of same phage-type following control of nasal carriers with neobacrin and soframycin. Lancet, II: 1168–1170, 1961.

72. JENNISON, R. F. and KOMROWER, G. M.: Effect of an antibacterial nasal cream on nasal colonization and infection in the newborn. Brit. med. J., I: 89–92, 1961.

73. JESSEN, C.: Luftbårne mikroorganismer, forekomst og bekæmpelse. Thesis. G. E. C. Gads forlag. København 1955.

74. KAY, C. R.: Sepsis in home. Brit. med. J., I: 1048–1052, 1962.

75. KLEIN, J. O. and ROGERS, E. F. H.: Use of a nasal antibiotic cream during a nursery outbreak of staphylococcal disease. New Engl. J. Med., 260: 1012–1015, 1959.

76. KÅSS, A. and VOGELSANG, TH. M.: Staphylococcal studies in a pediatric clinic. 1. Carrier rate of pathogenic staphylococci. Årb. Univ. Bergen Med. Ser. 1954, No. 3, 1–26.

77. LAURELL, G. and WALLMARK, G.: Studies on Staphylococcus aureus pyogenes in a children's hospital. I. Incidence in patients and staff. Acta path. microbiol. scand., 32: 424–431, 1953.

78. LAURELL, G. and WALLMARK, G.: Studies on Staphylococcus aureus pyogenes in a children's hospital. III. Results of phage typing and tests for penicillin resistance of 2474 strains isolated from patients and staff. Acta path. microbiol. scand., 32: 438–447, 1953.

79. LAWRIE, P. and JONES, B.: Hexachlorophene soap. Effect on the bacterial flora of the hands. Pharmaceut. J., 168: 288–290, 1952.

80. LOOSLI, C. G., LEMON, H. M., WISE, H. and Robertson, O. H.: Studies on the transmission and control of respiratory disease within army barracks. I. Hemolytic streptococcal contamination of the environment. J. infect. Dis., 82: 59–71, 1948.

81. LOVELL, D. L.: Skin bacteria: Their location with reference to skin sterilization. Surg., Gynec., Obstet., 80: 174–177, 1945.

82. LOVELL, D. L.: Preoperative skin preparation with reference to surface bacteria contaminants and resident flora. Surg. Clin. N. Amer., 26: 1053–1059, 1946.

83. LOWBURY, E. J. L. and LILLY, H. A.: Disinfection of the hands of surgeons and nurses. Brit. med. J., I: 1445–1450, 1960.

84. Lowbury, E. J. L., Lilly, H. A. and Bull, J. P.: Disinfection of the skin of operation sites. Brit. med. J., II: 1039–1044, 1960.

85. LOWBURY, E. J. L.: Skin disinfection. J. clin. Path., 14: 85–90, 1961.

86. LOWBURY, E. J. L., LILLY, H. A. and

BULL, J. P.: Disinfection of hands: Removal of resident bacteria. Brit. med. J., I: 1251–1256, 1963.

87. LUND, F.: Forekomsten af kemoresistente stafylokokker på hospitalsafdelinger. Thesis. Dansk videnskabs forlag A/S Copenhagen 1956.

88. MANFIELD, P. A., SHOOTER, R. A. and LIDWELL, O. M.: Nasal staphylococci and sepsis in newborn babies. Brit. med. J., I: 1098–1099, 1960.

89. MARTIN, T. D. M. and WHITEHEAD, J. E. M.: Carriage of penicillin-resistant Staph. pyogenes in healthy adults. Brit. med. J., I: 173–175, 1949.

90. MARTIN, W. J., NICHOLS, D. R. and HENDERSON, E. D.: The problem of management of nasal carriers of staphylococci. Proc. Mayo Clin., 35: 282–292, 1960.

91. MARTYN, G.: Staphylococci in the newborn. Their coagulase production and resistance to penicillin and streptomycin. Brit. med. J., I: 710–712, 1949.

92. MATTHIAS, J. Q., SHOOTER, R. A. and WILLIAMS, R. E. O.: Staphylococcus aureus in the fæces of hospital patients. Lancet, I: 1172–1173, 1957.

93. MÄKELÄ, P.: Studies on Staphylococcus aureus in Helsinki. The distribution of phage types and antibiograms, and their association with certain other characteristics. Ann. Med. exp. Biol. Fenn., 38: Suppl. 1, 1960.

94. MCDONALD, S. and TIMBURY, M. C.: Unusual outbreak of staphylococcal postoperative wound infection. Lancet, II: 863–864, 1957.

95. MCNEILL, I. F., PORTER, I. A. and GREEN, C. A.: Staphylococcal infection in a surgical ward. A three month study. Brit. med. J., II: 798–802. 1961.

96. MCQUADE, A. B. and SUTHERLAND, W. J. A.: An improved device for sampling bacterial populations on blankets. J. Hyg., Camb., 58: 157–158, 1960.

97. MILES, A. A., WILLIAMS, R. E. O. and CLAYTON-COOPER, B.: The carriage of Staphylococcus (pyogenes) aureus in man and its relation to wound infection. J. Path. Bact., 56: 513–524, 1944.

98. MOSS, B., SQUIRE, J. R., TOPLEY, E. and JOHNSTON, C. M.: Nose and skin carriage of Staphylococcus aureus in patients receiving penicillin. Lancet, I: 320–324, 1948.

99. NOBLE, W. C.: The dispersal of staphylococci in hospital wards. J. clin. Path., 15: 552–558, 1962.

100. OEDING, P.: Agglutinability of pyogenic staphylococci at various conditions. Acta path. microbiol. scand., 41: 310–324, 1957.

101. PLUECKHAHN, V. D.: The staphylococcus and the newborn child. Brit. med. J., II: 779–785, 1961.

102. PORTER, I. A., MILLER, I. T., MCNEILL, I. F. and GREEN, C. A.: Effect of topical framycetin on staphylococcal nasal carriage. Brit. med. J., I: 1515–1518, 1963.

103. PRICE, P. B.: The bacteriology of normal skin; a new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. J. infect. Dis., 63: 301–318, 1938.

104. PRICE, P. B.: Disinfection of the skin. Quart. Rev. Surg., 8: 263–271, 1951.

105. RAMMELKAMP, C. H.: Treatment of nasal carriers of staphylococci. Lancet, I: 112, 1961.

106. REBER, H., BIRCHER, J. and GRUMBACH, P.: Zur chirurgischen Händedesinfektion mit Hexachlorophen. Schweiz. Z. allg. Path. Bakt., 23: 581–586, 1960.

107. RIDLEY, M.: Perineal carriage of Staph. aureus. Brit. med. J., I: 270–273, 1959.

108. ROODYN, L.: Staphylococcal infections in general practice. Brit. med. J., II: 1322–1325, 1954.

109. ROUNTREE, P. M. and BARBOUR, R. G. H.: Nasal carrier rates of Staphylococcus pyogenes in hospital nurses. J. Path. Bact., 63: 313–324, 1951.

110. ROUNTREE, P. M., LOEWENTHAL, J., TEDDER, E. and GYE, R.: Staphylococcal wound infection: the use of neomycin and chlorhexidine ("Naseptin") nasal cream in its control. Med. J. Aust., II: 367–370, 1962.

111. RUBBO, S. D. and BENJAMIN, M.: Transmission of haemolytic streptococci. J. Hyg., Camb., 51: 278–292, 1953.

112. RUBBO, S. D. and DIXSON, S.: A contact-plate technique for determining bacterial contamination of fabrics. Lancet, II: 394–397, 1960.

113. RUBBO, S. D.: Treatment of nasal carriers of staphylococci. Lancet, I: 561, 1961.

114. RUBBO, S. D.: The role of textiles in hospital cross-infection. Pp. 231–250 in infection in hospitals. Epidemiology and control. Ed. Williams, R. E. O. and Shooter, R. A.' Blackwell Scientific Publications, Oxford, 1963.

115. SHEDDEN, W. I. H., POTTER, C. W. and BRUCE, A. M.: Framycetin and the Staphylococcus. Brit. med. J., II: 499, 1963.

116. SHOOTER, R. A., SMITH, M. A. and HUNTER, C. J. W.: A study of surgical masks. Brit. J. Surg., 47: 246–249, 1959.

117. SIBONI, K. E.: Staphylococcal endemia and prophylaxis. Thesis. Munksgaard. Copenhagen 1960.

118. SMYLIE, H. G., WEBSTER, C. U. and BRUCE, M. L.: "Phisohex" and safer surgery. Brit. med. J., II: 606–609, 1959.

119. SNEDECOR, G. W.: Statistical methods applied to experiments in agriculture and biology. Iowa State University press, Ames, Iowa, U.S.A., 1956.

120. STEEN, J. A. and ULSTRUP, J. C.: Nosocomial staphylococcal infections. I. Possible importance of nasal carriers in a maternity ward. J. Oslo Cy Hosp., 10: 41–49, 1960.

121. STOKES, E. J. and MILNE, S. E.: Effect of Naseptin cream prophylaxis on staphylococcal infection in adult surgical wards and infant nurseries. J. Hyg., Camb., 60: 209–215, 1962.

122. STORY, P.: Testing of skin disinfectants. Brit. med. J., II: 1128–1130, 1952.

123. STRATFORD, B., RUBBO, S. D., CHRISTIE, R. and DIXSON, S.: Treatment of the nasal carrier of Staphylococcus aureus with Framycetin and other antibacterials. Lancet, II: 1225–1227, 1960.

124. THAYSEN, E. HESS, ERIKSEN, K. RIEWERTS and ROSENDAL, K.: Cutane stafylokokinfektioner i en medicinsk afdeling. Ugeskr. Læg., 119: 1381–1388, 1957.

125. THOMAS, C. G. A. and GRIFFITHS, P. D.: Air-borne staphylococci and the control of hospital cross-infection. Guy's Hosp. Rep., 110: 76–86, 1961.

126. TULLOCH, L. G.: Nasal carriage in staphylococcal skin infections. Brit. med. J., II: 912–913, 1954.

127. TULLOCH, L. G., ALDER, V. G. and GILLESPIE, W. A.: Treatment of chronic furunculosis. Brit. med. J., II: 354–356, 1960.

128. TUNEWALL, G. and ERICSSON, H.: Sensitivity tests by disc method as guide for chemotherapy. Antibiot. and Chemother., 4: 886–893, 1954.

129. ULSTRUP, C. and Ødegaard, A.: Effect of methicillin spray on staphylococcal colonisation and lesions in a nursery. Lancet, II: 1227–1229, 1961.

130. VALENTINE, F. C. O. and HALL-SMITH, S. P.: Superficial staphylococcal infection. Lancet, II: 351–354, 1952.

131. VANBREUSEGHEM, R.: Staphylotoxine et staphylocoagulase. C. R. Soc. Biol. (Paris), 116: 650–652, 1934.

132. VARGA, D. T. and WHITE, A.: Suppression of nasal, skin and aerial staphylococci by nasal application of methicillin. J. clin. Invest., 40: 2209–2214, 1961.

133. VOGELSANG, TH. M.: The incidence of penicillin-resistant pathogenic staphylococci isolated from the upper respiratory tract of young healthy persons. Acta path. microbiol. scand., 39: 363–367, 1951.

134. VOGELSANG, TH. M.: Staphylococcal studies in hospital staffs. I. Carrier rates of pathogenic staphylococci. Acta path. microbiol. scand., 33: 294–300, 1953.

135. VOGELSANG, TH. M.: Staphylococcal

studies in hospital staffs. III. Bacteriophage typing. Acta path. microbiol. scand., 33: 435—448, 1953.

136. Vogelsang, Th. M.: Personal communication. 1962.

137. Vogelsang, Th. M. and Bøe, J.: A long-term staphylococcal study. Acta path. microbiol. scand., 54: 225—240, 1962.

138. Vogelsang, Th. M.: Staphylococcal studies in a maternity hospital. Årb. Univ. Bergen Med. Ser. 1963, No. 1, 1—36.

139. Wallmark, G.: Bacteriophage typing of Staphylococcus aureus pyogenes. I. A report of the method and some experimental results. Acta path. microbiol. scand., 34: 57—67, 1954.

140. Weinstein, H. J.: The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. New Engl. J. Med., 260: 1303—1308, 1959.

141. Weinstein, H. J.: Control of nasal-staphylococcal-carrier states. New Engl. J. Med., 260: 1308—1310, 1959.

142. White, A., Hemmerly, T., Martin, M. P. and Knight, V.: Studies on the origin of drug-resistant staphylococci in a mental hospital. Amer. J. Med., 27: 26—39, 1959.

143. White, A.: Quantitative studies of nasal carriers of staphylococci among hospitalized patients. J. clin. Invest., 40: 23—30, 1961.

144. White, A.: Relation between quantitative nasal cultures and dissemination of staphylococci. J. Lab. clin. Med., 58: 273—277, 1961.

145. Williams, R. E. O.: Skin and nose carriage of bacteriophage types of Staph. aureus. J. Path. Bact., 58: 259—268, 1946.

146. Williams, R. E. O. and Rippon, J. E.: Bacteriophage typing of Staphylococcus aureus. J. Hyg., Camb., 50: 320—353, 1952.

147. Williams, R. E. O., Lidwell, O. M. and Hirch, A.: The bacterial flora of the air of occupied rooms. J. Hyg., Camb., 54: 512—523, 1956.

148. Williams, R. E. O., Jevons, M. P., Shooter, R. A., Hunter, C. J. W., Girling, J. A., Griffiths, J. D. and Taylor, G. W.: Nasal staphylococci and sepsis in hospital patients. Brit. med. J., II: 658—662, 1959.

149. Williams, R. E. O., Blowers, R., Garrod, L. P. and Shooter, R. A.: Hospital infection. Causes and prevention. Lloyd-Luke Ltd., London, 1960.

Appendix table. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
1	1	11,040	0.12	1.12	2.46	1.14	3.60	280.0	3.300
F	2	5,320	<0.04	0.56	<0.02	0.96	0.96	20.0	2.900
14	3	14,840	0.80	0.36	6.54	5.54	12.08	280.0	10.100
2	4	188	<0.04	<0.02	0.08	<0.02	0.08	<0.5	0.100
F	5	448	<0.04	0.16	0.24	<0.02	0.24	0.5	0.200
72	6	192	<0.04	<0.02	0.24	<0.02	0.24	<0.5	0.200
3	7	264	<0.04	0.28	0.28	0.18	0.46	0.5	0.200
M	8	120	<0.04	0.28	0.08	<0.02	0.08	<0.5	0.100
58	9	156	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
4	10	3,040	<0.04	<0.02	6.24	<0.02	6.24	60.0	0.500
F	11	4,240	<0.04	1.16	0.96	<0.02	0.96	40.0	0.800
24	12	4,600	<0.04	0.76	2.64	<0.02	2.64	40.0	0.800
5	13	40	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
M	14	28	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.050
53	15	12	<0.04	<0.02	0.06	<0.02	0.06	<0.5	0.025
6	16	104	0.04	<0.02	0.08	<0.02	0.08	<0.5	0.200
F	17	120	<0.04	0.12	<0.02	<0.02	<0.02	<0.5	0.100
35	18	728	2.00	0.08	0.48	<0.02	0.48	1.5	0.300
7	19	200	1.32	0.04	0.08	<0.02	0.08	3.5	0.500
F	20	260	49.60	<0.02	<0.02	<0.02	<0.02	0.5	0.800
14	21	212	12.32	0.68	0.24	0.56	0.80	14.0	1.400
8	22	4,120	0.12	0.96	3.26	0.10	3.36	28.0	1.600
M	23	1,240	<0.04	0.04	0.32	<0.02	0.32	42.0	2.800
17	24	4,640	<0.04	14.40	<0.02	8.08	8.08	22.0	5.100
9	25	8,640	<0.04	0.72	<0.02	0.64	0.64	100.0	0.900
M	26	2,480	0.04	8.82	0.64	0.64	1.28	60.0	1.400
48	27	2,080	<0.04	4.08	1.44	0.24	1.68	40.0	0.500
10	28	9,240	0.32	0.60	6.00	1.44	7.44	380.0	4.900
F	29	2,840	<0.04	0.04	1.08	0.08	1.16	100.0	2.900
60	30	3,240	1.20	0.16	0.48	0.16	0.64	100.0	4.100

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
11	31	16	0.08	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
M	32	28	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
29	33	20	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
12	34	1,264	<0.04	0.08	2.40	0.08	2.48	40.0	0.200
F	35	5,360	0.04	0.12	2.16	<0.02	2.16	20.0	0.400
47	36	8,800	<0.04	4.04	0.08	2.48	2.56	60.0	1.300
13	37	360	0.20	0.84	0.08	<0.02	0.08	<0.5	0.200
M	38	120	0.24	<0.02	0.16	0.16	0.32	1.0	0.200
16	39	120	<0.04	0.16	0.08	<0.02	0.08	<0.5	0.100
14	40	1,240	<0.04	0.32	0.24	<0.02	0.24	8.5	1.100
F	41	1,020	<0.04	0.12	1.52	<0.02	1.52	13.5	1.400
43	42	4,400	<0.04	0.72	5.84	<0.02	5.84	32.0	3.200
15	43	1;120	<0.04	0.64	0.08	<0.02	0.08	2.0	1.400
M	44	440	<0.04	0.08	1.12	0.08	1.20	1.5	0.500
19	45	1,360	0.04	0.16	1.58	<0.02	1.58	6.5	1.200
16	46	3,480	0.24	0.12	12.84	0.64	13.48	40.0	3.600
F	47	2,880	0.96	3.76	21.68	0.24	21.92	40.0	4.100
64	48	5,680	0.52	0.24	2.08	<0.02	2.08	40.0	2.700
17	49	16,240	<0.04	0.20	2.88	0.16	3.04	10.0	11.500
F	50	14,240	<0.04	2.04	432.00	25.60	457.60	1,358.0	19.400
65	51	22,560	0.08	1.24	20.80	0.32	21.12	1,120.0	14.600
18	52	40	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
M	53	40	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
16	54	20	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
19	55	280	<0.04	<0.02	0.06	<0.02	0.06	0.5	0.100
M	56	464	<0.04	0.08	0.06	<0.02	0.06	1.5	0.100
42	57	320	<0.04	<0.02	0.10	<0.02	0.10	0.5	0.100
20	58	2,400	<0.04	5.60	0.36	3.24	3.60	12.0	4.900
M	59	2,756	<0.04	3.36	2.08	0.14	2.20	6.5	3.700
52	60	1,280	<0.04	2.04	0.26	0.10	0.36	7.5	3.300

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
21	61	4,328	<0.04	0.24	0.08	2.44	2.52	46.0	6.200
M	62	8,640	<0.04	12.72	0.68	3.04	3.72	78.0	3.800
16	63	8,200	<0.04	2.80	0.16	1.60	1.76	64.0	4.600
22	64	792	0.32	0.16	0.04	0.04	0.08	8.0	0.800
F	65	1,240	0.28	0.24	0.04	0.68	0.72	8.0	0.900
20	66	1,268	0.24	0.12	0.16	<0.02	0.16	12.0	1.400
23	67	174	<0.04	<0.02	0.08	<0.02	0.08	14.0	0.200
M	68	124	0.04	0.04	0.24	<0.02	0.24	1.5	0.500
21	69	120	<0.04	0.04	0.16	<0.02	0.16	0.5	0.200
24	70	12,960	<0.04	1.28	0.56	0.08	0.64	10.0	0.600
M	71	17,120	<0.04	17.52	0.40	2.00	2.40	28.0	0.400
65	72	9,440	<0.04	2.80	2.64	0.40	3.04	56.0	0.500
25	73	3,040	0.10	0.16	0.26	0.06	0.32	100.0	2.000
F	74	1,004	0.12	5.44	<0.02	0.08	0.08	40.0	1.000
48	75	1,620	0.88	1.88	0.90	0.86	1.76	16.0	1.300
26	76	800	0.04	0.52	0.22	0.06	0.28	6.5	2.000
F	77	152	<0.04	<0.02	0.04	<0.02	0.04	1.5	0.700
30	78	344	<0.04	0.04	0.30	0.02	0.32	12.0	0.500
27	79	18,840	0.56	9.28	101.00	22.00	123.00	480.0	21.400
M	80	18,120	<0.04	31.00	11.60	2.40	14.00	100.0	16.300
29	81	14,040	<0.04	6.00	5.00	1.00	6.00	400.0	10.400
28	82	80	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
F	83	92	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
24	84	112	<0.04	0.56	0.20	<0.02	0.20	0.5	0.025
29	85	1,320	<0.04	0.04	0.24	0.32	0.56	4.0	1.400
F	86	4,640	<0.04	0.08	0.18	0.14	0.32	20.0	1.200
70	87	680	<0.04	0.04	1.04	<0.02	1.04	2.0	1.300
30	88	360	0.04	0.08	0.72	<0.02	0.72	4.0	0.200
F	89	160	<0.04	<0.02	0.08	<0.02	0.08	2.0	0.200
33	90	992	<0.04	0.08	0.16	0.08	0.24	6.0	1.200

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
31	91	1,280	<0.04	4.80	1.04	<0.02	1.04	14.0	0.500
F	92	620	0.28	0.16	0.24	0.08	0.32	4.0	0.700
55	93	4,240	0.16	0.92	3.24	0.16	3.40	16.0	1.800
32	94	176	<0.04	0.08	<0.02	<0.02	<0.02	4.0	0.100
M	95	216	<0.04	0.04	0.08	<0.02	0.08	1.0	0.025
18	96	180	<0.04	0.12	0.28	0.04	0.32	2.0	0.100
33	97	400	0.72	0.04	0.08	<0.02	0.08	4.0	0.100
F	98	880	1.64	0.08	0.16	0.08	0.24	4.0	0.100
43	99	1,000	0.12	0.04	0.04	0.20	0.24	14.0	0.300
34	100	108	<0.04	0.04	0.48	<0.02	0.48	2.0	0.100
F	101	24	<0.04	<0.02	0.20	<0.02	0.20	<0.5	0.100
64	102	108	<0.04	<0.02	0.84	0.04	0.88	1.0	0.100
35	103	320	<0.04	0.08	<0.02	0.04	0.04	6.0	1.100
F	104	2,040	<0.04	2.04	1.44	2.20	3.64	100.0	4.900
15	105	320	<0.04	0.08	0.32	<0.02	0.32	2.0	2.400
36	106	2,920	0.16	2.44	2.40	2.48	4.88	172.0	0.700
F	107	1,600	0.68	2.88	0.28	<0.02	0.28	12.0	0.200
61	108	640	0.16	0.04	0.04	<0.02	0.04	2.0	0.400
37	109	2	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
F	110	1	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
17	111	3	0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
38	112	12	<0.04	<0.02	0.02	<0.02	0.02	<0.5	0.100
F	113	28	<0.04	<0.02	0.16	<0.02	0.16	<0.5	0.100
41	114	48	<0.04	<0.02	0.02	<0.02	0.02	1.0	<0.025
39	115	2	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
M	116	1	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
45	117	1	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
40	118	32,080	3.28	25.60	56.00	12.40	68.40	720.0	39.800
M	119	22,520	1.20	41.00	24.00	0.48	24.48	360.0	14.400
52	120	14,200	1.24	37.00	4.08	26.00	30.08	820.0	13.200

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
41	121	3,440	<0.04	0.40	2.24	<0.02	2.24	2.0	1.500
F	122	3,920	<0.04	0.12	9.52	0.60	10.12	12.0	1.900
75	123	2,800	<0.04	0.08	1.44	<0.02	1.44	4.0	1.200
42	124	92	0.08	0.16	0.02	0.02	0.04	4.0	0.300
M	125	208	<0.04	0.12	0.12	0.62	0.74	6.0	0.400
57	126	1,120	0.24	0.24	0.76	<0.02	0.76	2.0	0.400
43	127	9,200	0.16	1.40	5.00	0.12	5.12	60.0	2.000
F	128	1,440	<0.04	0.88	4.80	0.96	5.76	12.0	1.200
66	129	2,400	<0.04	0.04	24.00	0.40	24.40	12.0	4.000
44	130	4,040	<0.04	0.68	1.76	1.64	3.40	36.0	2.800
M	131	880	0.32	1.46	0.12	0.08	0.20	12.0	1.100
28	132	1,120	<0.04	0.48	22.80	0.32	23.12	35.0	2.400
45	133	2,080	<0.04	0.96	3.60	21.20	24.80	122.0	2.500
M	134	2,240	<0.04	0.24	0.36	1.04	1.40	60.0	3.700
24	135	880	<0.04	2.44	1.64	<0.02	1.64	32.0	2.200
46	136	2,040	<0.04	0.04	0.24	<0.02	0.24	4.0	0.300
M	137	1,800	<0.04	0.08	1.04	<0.02	1.04	20.0	0.900
24	138	4,120	<0.04	0.04	<0.02	1.00	1.00	60.0	0.800
47	139	240	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
M	140	1,200	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
70	141	2,840	<0.04	<0.02	0.32	0.04	0.36	8.0	0.500
48	142	1,200	<0.04	0.12	1.32	0.32	1.64	28.0	6.400
F	143	8,240	<0.04	1.26	4.42	0.12	4.54	46.0	9.000
50	144	9,200	<0.04	1.92	1.60	0.04	1.64	10.0	3.100
49	145	2,800	2.04	4.00	11.00	0.88	11.88	52.0	0.700
M	146	5,440	0.20	6.00	0.92	0.60	1.52	71.0	1.100
15	147	480	0.32	1.20	0.04	0.56	0.60	10.0	0.900
50	148	2,480	<0.04	0.88	0.04	8.16	8.20	42.0	1.300
F	149	840	<0.04	2.84	0.32	<0.02	0.32	22.0	0.600
47	150	3,420	<0.04	0.16	0.32	<0.02	0.32	15.0	0.900

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
51	151	16	<0.04	<0.02	0.02	<0.02	0.02	0.5	0.100
M	152	8	<0.04	<0.02	0.02	<0.02	0.02	<0.5	0.025
20	153	8	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
52	154	880	1.60	0.12	0.28	0.28	0.56	0.5	2.200
F	155	1,640	0.80	2.88	1.06	0.02	1.08	7.5	0.700
57	156	800	<0.04	0.08	0.14	<0.02	0.14	0.5	1.300
53	157	800	<0.04	0.24	0.36	<0.02	0.36	1.0	0.200
M	158	160	<0.04	0.16	0.16	0.08	0.24	3.0	0.300
73	159	120	<0.04	<0.02	0.20	<0.02	0.20	1.5	0.200
54	160	920	<0.04	1.32	8.12	2.62	10.74	11.0	1.100
F	161	120	<0.04	<0.02	0.20	0.02	0.22	3.0	0.700
73	162	400	<0.04	1.24	2.08	<0.02	2.08	12.0	0.800
55	163	640	<0.04	0.84	1.44	0.42	1.86	8.0	0.600
F	164	320	<0.04	0.18	3.80	<0.02	3.80	7.0	0.900
23	165	3,960	<0.04	7.42	3.48	1.68	5.16	50.0	1.000
56	166	80	<0.04	<0.02	0.12	<0.02	0.12	<0.5	0.300
M	167	16	<0.04	<0.02	0.02	<0.02	0.02	<0.5	0.050
57	168	80	<0.04	0.10	<0.02	0.24	0.24	1.0	0.300
57	169	40	0.88	<0.02	0.32	0.04	0.36	1.5	0.200
F	170	48	1.60	0.02	0.64	0.02	0.66	3.0	0.100
69	171	20	0.68	<0.02	0.02	<0.02	0.02	<0.5	0.100
58	172	2	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
M	173	5	<0.04	0.02	0.04	<0.02	0.04	<0.5	<0.025
67	174	7	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
59	175	160	0.04	1.04	4.22	<0.02	4.22	14.5	0.200
F	176	1,160	1.24	0.44	1.08	<0.02	1.08	2.5	0.300
72	177	360	<0.04	0.12	0.16	0.04	0.20	0.5	1.000
60	178	280	<0.04	0.22	0.48	<0.02	0.48	8.5	0.700
M	179	120	0.08	1.04	0.58	<0.02	0.58	0.5	0.500
31	180	240	<0.04	0.06	0.08	<0.02	0.08	0.5	0.600

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
61	181	332	<0.04	0.46	0.14	0.18	0.32	19.5	1.400
M	182	640	<0.04	0.52	1.62	0.20	1.82	50.5	3.800
62	183	1,160	<0.04	0.60	0.48	0.04	0.52	23.0	1.600
62	184	364	<0.04	0.04	0.22	<0.02	0.22	3.5	0.100
M	185	64	<0.04	<0.02	0.16	<0.02	0.16	0.5	0.100
52	186	400	<0.04	0.94	1.06	0.02	1.08	0.5	0.600
63	187	880	<0.04	<0.02	<0.02	<0.02	<0.02	2.5	0.100
F	188	600	<0.04	<0.02	0.04	<0.02	0.04	<0.5	<0.025
51	189	920	<0.04	<0.02	0.06	<0.02	0.06	2.5	0.200
64	190	240	<0.04	0.08	0.22	0.12	0.34	<0.5	0.600
M	191	364	<0.04	0.08	0.08	<0.02	0.08	1.5	0.400
27	192	652	<0.04	0.82	0.64	<0.02	0.64	<0.5	0.500
65	193	20	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
M	194	60	<0.04	<0.02	<0.02	0.06	0.06	<0.5	0.050
59	195	28	<0.04	<0.02	0.02	<0.02	0.02	<0.5	<0.025
66	196	480	<0.04	0.14	0.86	0.94	1.80	31.0	0.900
F	197	1,640	<0.04	0.42	0.98	0.54	1.52	12.5	0.300
23	198	920	0.04	0.14	1.38	0.06	1.44	9.5	0.300
67	199	3,240	0.20	7.94	1.02	8.26	9.28	28.5	5.700
F	200	1,800	0.20	0.42	1.68	18.62	20.30	23.5	2.100
74	201	4,040	0.04	6.22	16.58	8.54	25.12	46.5	2.700
68	202	360	<0.04	<0.02	<0.02	0.04	0.04	<0.5	0.025
M	203	280	<0.04	0.04	<0.02	<0.02	<0.02	<0.5	0.025
52	204	800	<0.04	<0.02	0.14	<0.02	0.14	<0.5	0.025
69	205	400	<0.04	<0.02	0.66	0.08	0.74	1.5	1.500
F	206	840	<0.04	0.06	<0.02	3.42	3.42	1.5	1.300
73	207	1,560	<0.04	<0.02	0.46	<0.02	0.46	1.5	1.100
70	208	24	<0.04	<0.02	0.04	<0.02	0.04	<0.5	<0.025
M	209	120	<0.04	0.18	<0.02	0.54	0.54	1.0	0.200
48	210	20	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
71	211	2,400	<0.04	<0.02	0.06	0.62	0.68	2.5	1.500
F	212	12,440	0.04	0.92	10.22	0.12	10.34	65.5	5.600
72	213	3,120	<0.04	0.24	0.46	0.06	0.52	30.0	1.100
72	214	480	<0.04	<0.02	0.28	<0.02	0.28	2.5	0.700
M	215	3,600	<0.04	14.00	14.00	3.00	17.00	45.0	4.300
66	216	1,960	0.08	0.32	0.88	<0.02	0.88	3.5	1.200
73	217	360	<0.04	0.16	0.06	<0.02	0.06	<0.5	0.400
F	218	1,840	<0.04	0.36	8.84	<0.02	8.84	8.5	1.200
58	219	640	<0.04	<0.02	0.10	<0.02	0.10	1.0	0.200
74	220	680	<0.04	1.36	2.52	<0.02	2.52	2.5	0.400
M	221	1,080	<0.04	0.12	0.38	<0.02	0.38	11.5	0.200
40	222	2,800	0.04	0.42	26.00	0.06	26.06	63.0	0.600
75	223	680	<0.04	0.54	7.04	0.02	7.06	20.0	1.700
F	224	840	<0.04	0.04	0.74	0.12	0.86	22.5	2.600
45	225	4,080	<0.04	0.12	0.32	3.84	4.16	20.0	2.100
76	226	2,400	<0.04	1.62	121.00	0.48	121.48	190.0	6.300
F	227	2,800	<0.04	0.16	11.00	0.06	11.06	122.0	7.500
52	228	3,240	0.08	1.66	6.18	0.42	6.60	55.5	6.200
77	229	680	<0.04	0.06	0.12	<0.02	0.12	1.0	0.300
F	230	108	<0.04	<0.02	0.08	0.22	0.30	<0.5	0.100
41	231	292	<0.04	<0.02	0.08	<0.02	0.08	<0.5	0.100
78	232	920	0.04	0.02	<0.02	0.16	0.16	<0.5	0.025
F	233	480	<0.04	0.04	<0.02	0.12	0.12	<0.5	0.025
44	234	120	<0.04	0.10	0.04	<0.02	0.04	0.5	0.025
79	235	11,240	<0.04	16.00	69.00	10.00	79.00	89.0	29.400
M	236	14,400	<0.04	16.00	191.00	48.00	239.00	155.0	43.400
51	237	14,320	<0.04	4.00	48.00	4.00	52.00	51.5	21.900
80	238	5,080	0.04	1.04	16.66	9.62	26.28	186.0	7.200
F	239	4,200	<0.04	0.90	4.98	2.80	7.78	31.5	3.800
65	240	6,080	0.08	2.62	129.00	6.00	135.00	149.0	5.600

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
81	241	80	<0.04	<0.02	0.08	0.66	0.74	2.5	0.300
F	242	40	<0.04	0.16	2.62	0.06	2.68	1.5	0.500
61	243	24	<0.04	<0.02	0.16	<0.02	0.16	<0.5	0.200
82	244	608	<0.04	0.48	3.00	3.00	6.00	7.0	1.000
M	245	448	<0.04	0.62	0.84	<0.02	0.84	1.5	1.200
45	246	600	<0.04	1.02	0.08	<0.02	0.08	<0.5	0.300
83	247	680	0.04	0.68	0.20	<0.02	0.20	1.0	0.075
F	248	2,800	<0.04	0.02	<0.02	<0.02	<0.02	<0.5	<0.025
64	249	160	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
84	250	520	<0.04	0.08	0.44	4.22	4.66	10.5	2.500
M	251	680	<0.04	0.22	2.46	7.22	9.68	16.5	2.900
33	252	3,240	<0.04	3.68	0.42	1.82	2.24	8.5	7.300
85	253	320	<0.04	<0.02	0.06	<0.02	0.06	<0.5	0.300
F	254	2,800	<0.04	0.04	0.10	<0.02	0.10	1.5	0.200
49	255	640	<0.04	<0.02	0.72	0.18	0.90	9.5	0.100
86	256	560	0.04	0.10	<0.02	0.92	0.92	28.5	2.000
M	257	640	<0.04	<0.02	0.54	0.26	0.80	11.5	2.400
42	258	280	0.12	0.06	0.04	0.06	0.10	3.5	1.000
87	259	2,080	<0.04	<0.02	1.14	<0.02	1.14	4.5	2.000
M	260	3,640	<0.04	2.70	6.66	0.24	6.90	83.0	1.700
57	261	2,880	<0.04	0.44	6.22	0.16	6.38	16.5	2.100
88	262	4	<0.04	<0.02	0.04	<0.02	0.04	<0.5	<0.025
M	263	28	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	<0.025
49	264	4	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.025
89	265	4,800	2.48	1.06	61.00	2.00	63.00	34.5	7.800
F	266	5,040	1.24	0.86	39.00	3.00	42.00	71.0	7.500
16	267	2,480	0.84	0.42	7.00	<0.02	7.00	9.5	8.700
90	268	7,240	2.04	0.62	49.00	1.42	50.42	171.0	7.600
F	269	6,960	1.92	<0.02	1.62	6.24	7.86	36.0	10.000
17	270	1,040	1.76	0.02	36.00	0.14	36.14	35.5	7.900

Appendix table cont. *Skin contamination and aerial dissemination of staphylococci; 100 nasal carriers. Estimated no. of bacteria (no. of colonies × dil.; in thousands).*

Pat. no., sex, age (yrs.)	Exp. no.	Nose	Throat	Upper lip	Fingers			Hands	Air contamination (bed making)
					Left	Right	Sum		
91	271	28,440	0.80	2.22	2.06	4.82	6.88	50.5	16.700
F	272	5,280	<0.04	0.82	2.70	2.38	5.08	19.5	17.100
54	273	22,440	<0.04	0.92	29.00	12.00	41.00	350.0	13.700
92	274	752	<0.04	1.64	1.04	<0.02	1.04	6.0	1.200
M	275	1,520	0.24	0.72	1.16	<0.02	1.16	11.5	1.300
60	276	360	0.04	1.24	0.28	<0.02	0.28	<0.5	0.400
93	277	14,000	<0.04	0.06	0.12	<0.02	0.12	8.5	1.300
M	278	7,640	<0.04	0.06	0.02	29.00	29.02	21.0	1.800
22	279	1,040	<0.04	2.22	3.00	<0.02	3.00	9.5	0.800
94	280	7,640	<0.04	0.04	37.00	0.08	37.08	70.5	5.500
M	281	5,200	<0.04	0.14	76.00	1.18	77.18	34.5	2.800
46	282	12,080	0.16	2.32	131.00	4.00	135.00	112.0	3.800
95	283	200	1.84	2.80	0.86	0.16	1.02	4.5	0.400
M	284	160	0.42	0.02	1.24	0.16	1.40	1.5	0.300
64	285	400	0.42	0.96	0.36	0.02	0.36	1.5	0.300
96	286	2,880	0.04	0.06	33.00	5.00	38.00	70.0	2.400
F	287	1,280	0.12	0.42	5.00	3.00	8.00	40.0	5.700
64	288	3,240	0.08	0.38	53.00	8.00	61.00	200.0	4.000
97	289	520	0.20	0.20	0.10	0.16	0.26	13.5	0.300
M	290	440	0.32	0.56	0.62	0.44	1.06	8.5	0.400
44	291	1,560	<0.04	0.10	0.06	0.10	0.16	11.0	0.300
98	292	40	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
F	293	164	<0.04	<0.02	0.02	<0.02	0.02	<0.5	<0.025
60	294	320	<0.04	0.04	0.48	<0.02	0.48	<0.5	0.200
99	295	2,240	<0.04	0.06	0.06	0.02	0.08	4.5	0.400
M	296	320	<0.04	0.04	0.34	0.46	0.80	<0.5	0.600
75	297	2,040	<0.04	0.98	0.32	<0.02	0.32	1.5	0.300
100	298	920	<0.04	<0.02	0.12	<0.02	0.12	<0.5	0.100
F	299	1,700	<0.04	<0.02	<0.02	<0.02	<0.02	<0.5	0.100
77	300	160	<0.04	0.04	0.90	<0.02	0.90	0.5	0.500

FROM THE CENTRAL HOSPITAL, TAMPERE, FINLAND

EXPERIENCES IN THE USE OF DIRECT CURRENT COUNTERSHOCK IN THE TREATMENT OF CARDIAC ARRHYTHMIAS

By

M. Reinikainen, P. Koskinen, P. Pöntinen and L. Siitonen

TAMPERE 1965