J. Hyg., Camb. (1968), **66**, 567
Printed in Great Britain

Bacteriological aspects of air-conditioning plants

By W. WHYTE

Building Services Research Unit, University of Glasgow, Glasgow, W. 2, and the University Department of Bacteriology and Immunology, Western Infirmary, Glasgow, W. 1

(*Received* 11 *March* 1968)

It has been shown in hospitals that positive ventilation in operating theatres may cut the risk of sepsis (Blowers, Mason, Wallace & Walton, 1955; Shooter, Taylor, Ellis & Ross, 1956) and is of value in the treatment of burns (Lowbury, 1954). Many other zones in the hospital could benefit from the supply of clean air, e.g. ward areas, intensive care units and laboratories, and although it has yet to be proved that this would cut down the risk of cross-infection, it would seem beneficial. Ventilation may also be necessary owing to architectural requirements as in deep plan buildings and also to provide comfortable conditions. Ventilation systems are being provided more frequently in hospitals and there is a need for data on the hazards and usefulness of air-conditioning systems in providing clean air.

The Hairmyres Experimental Ward (Report, 1964) has given an excellent opportunity for the study of air-conditioning systems. The 'race track' ward is supplied by four ventilation plants, each different in design. The air-conditioning plant is supplied with fresh air or with a mixture of fresh air and air recirculated from the inside environment. The air-conditioning system is composed of a primary filter, preheater, fan, humidifier and cooling coils, secondary filter, heater battery and supply ducting.

Filtration efficiency. The Medical Research Council Sub-Committee for Operating Theatre Hygiene (Report, 1962) has suggested that filtration down to 5 μ should be adequate for filtration of the air supplied to operating theatres. This recommendation was based on work by Blowers & Crew (1960), who showed that this standard was quite adequate for a full fresh-air system. However, this conclusion has been questioned and no data exist on the efficiency of filtration appropriate to other parts of the hospital. The question of the efficiency of filtration for recirculating air has not been investigated. There is also the possibility that some filters could support the growth of bacteria.

Humidification. Attention has been brought to the bacteriological hazards in air-conditioning humidifiers which use recirculatory tanks (Blowers, Lidwell & Williams, 1962; Shaffer, 1963). Organisms from the pseudomonas, proteus and staphylococcus genera have been incriminated; the primary source of these organisms being the air passing through the sytsem. They may then multiply in the water and are carried into the atmosphere of the room being air conditioned.

Duct surfaces. It has been suggested that dust and bacteria building up inside the air-conditioning ducts may be a potential hazard.

In this study the potential hazards and uses of filters, humidifiers and ducts have been assessed.

MATERIALS AND METHODS

Air-conditioning equipment

At the Hairmyres Experimental Ward four ventilation plants were installed to serve the four zones of the ward. Since two of these plants are similar in construction only three plants were studied. These were those serving the Central Core, Ward and Intensive Care Areas. Built to supply different areas of the ward unit and to enable assessment of various designs, they were provided with different

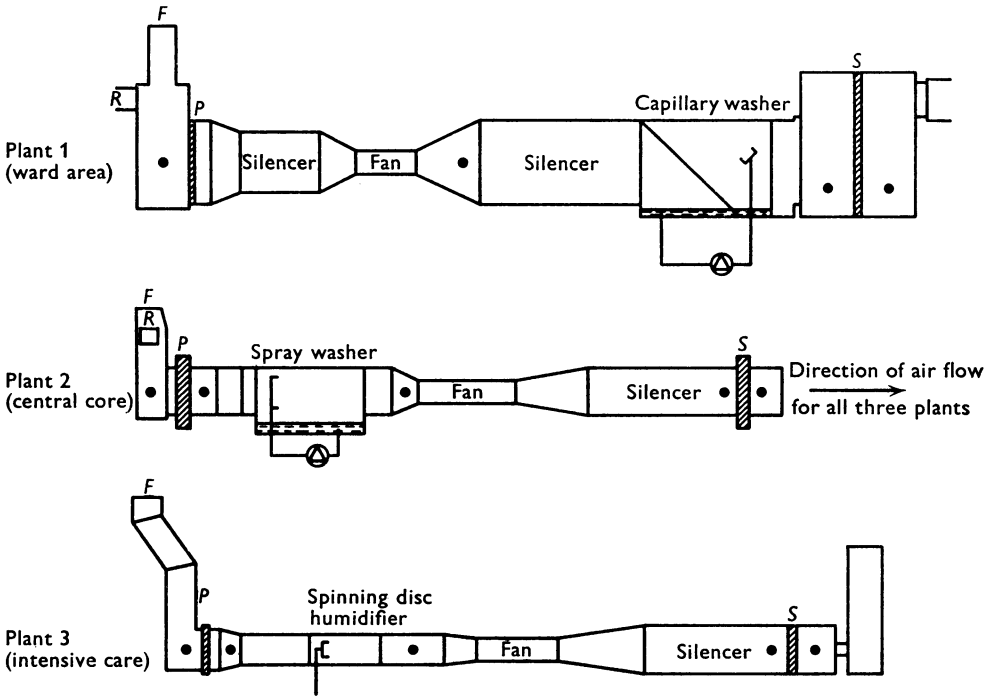

Fig. 1. Diagram of the three air-conditioning plants. ●, sampling point; *P*, primary filter; *S*, secondary filter; *F*, fresh-air duct; *R*, recirculatory duct.

equipment. The three plants are set out schematically in Fig. 1, and will be referred to as plants 1, 2 and 3. As can be seen, each plant has a different type of humidifier. These, as supplied by Copperad Ltd., were a capillary washer, a spray washer and a spinning disk humidifier.

The types of filters in each of the three plants studied are shown in Table 1. The dust-retaining efficiency under specific test specifications of these filters is given in Table 2. Also included are efficiency figures for the Vokes K 600 Kompak, the filter studied by Blowers & Crew (1960). The size distribution of the three dusts conforming to B.S. 2831 are as follows. Test Dust no. 1 includes particles

within the range 0·2 –2 μ in diameter, about 50 % by weight of these particles being over 0·5 μ. Sixty to seventy per cent by weight of the particles of Test Dust no. 2 are within the range 3·5–7 μ in size, 99·5 % of them finer than 13 μ and 2 % finer than 2·5 μ. In Test Dust no. 3, 60–80 % by weight of the particles are within the range 15–25 μ in size, 99·5 % finer than 35 μ and 2 % finer than 10 μ.

The air intakes were placed 23 ft. above ground level and situated towards the centre of the top of the building. The exhausts were 52 ft. from the intakes at each end of the top of the building.

Table 1. *Types of filter in the three air-conditioning plants studied*

Air-conditioning plant	Primary filter	Secondary filter
Plant 1 (ward area)	Vokes Supervee	Under full fresh air– Vokes Univee Grade C Under recirculation– Vokes Univee Grade A
Plant 2 (central core)	Vokes Miniroll Standard	Vokes Miniroll Standard
Plant 3 (intensive care)	Vokes Supervee	Vokes Univee Grade C

Table 2. *Dust retaining efficiency of filters (expressed as the percentage of dust retained by the filters)*

(Data derived from Mulcaster & Stokes 1966)

	B.S. 2831 specification		
Type of filter	Test Dust no. 1	Test Dust no. 2	Test Dust no. 3
Vokes Supervee	5–10	93·5	50·6
Vokes Univee Grade A	75–82	99·35	99·0
Vokes Univee Grade C	18–37	91·8	26·0
Vokes Miniroll Standard	10–30	87·5	68·0
Vokes K 600 Kompak	20–40	97·4	46·6

Bacteriological analysis of air

Bacteriological analysis of air was by means of Andersen samplers (Andersen, 1958). These were placed at the positions shown in Fig. 1, on their sides and facing the air stream. The entry cone usually supplied with this sampler was dispensed with, as this cone causes heavy loss of larger particles on the top sieve (May, 1964). A hinged lid was substituted. The samplers were loaded in an air-conditioned room, only the person filling the sampler being present. This person was capped, masked, gowned and wore sterile surgeon's gloves. The samplers were then placed in the ducts in their appropriate positions with the lids shut. The air-conditioning system was allowed to run for 10 min. in order to clear out any residual contamination. The lid was then pulled back and a 15 cu.ft. sample taken from all six samplers simultaneously. Isokinetic sampling was not considered necessary. Counts were made of the total number of micro-organisms, the total numbers of aerobic bacteria (henceforth known as total bacterial count), *Staphylococcus aureus* and *Clostridium welchii*. Phenolphthalein diphosphate medium (Barber & Kuper, 1951) was used for total micro-organism counts, total bacterial counts and for presumptive counts of *Staph. aureus*. The presumptive *Staph. aureus* were coagulase tested and phage typed.

The size distribution of the micro-organisms was calculated graphically. The percentage cumulated counts by Andersen stages were calculated on a 'less than stated size' for each sampler stage and were plotted on probability scale × 3 cycle log paper or probability scale × 2 cycle log paper, setting the plot point, in microns, as the $D_{50\%}$ of the next stage above. The values for 50 % cumulative particle impaction ($D_{50\%}$) per stage were obtained from published results (Kethley, Cown & Fincher, 1962) and given in Table 3 in terms of equivalent particle diameter, where the equivalent particle is the diameter of a sphere of unit density which has a settling rate in air equal to that of the particle in question.

Table 3. *Fifty per cent cumulative particle impaction per stage of an Andersen bacterial sampler*

Andersen stage	Size of 50% cumulative impaction (μ)
1	9·8
2	6·2
3	3·8
4	2·2
5	0·9

From the line of best fit drawn through the points the equivalent median diameter may be obtained by reading the particle size opposite 50%. The geometrical standard deviation (σg), which is the antilog of the slope of the distribution plot, may be derived directly from the graph by the relationship

$$\sigma g = \frac{84{\cdot}13\,\%\text{ size}}{50\,\%}(\mu) = \frac{50\,\%\text{ size}}{15{\cdot}87\,\%\text{ size}}(\mu).$$

A modification of the medium described by Lowbury & Lilly (1955) was used for the isolation of *Cl. welchii*. This consisted of two layers, a bottom layer of 12 ml. of agar base and a top layer of 15 ml. of supplemented agar. These proportions were finally arrived at as it was found that in order to have the even layer necessary for the Andersen sampler a large top layer was required. The agar base consisted of 2 % Evans peptone with 1·5 % 'Ion agar'. 1·5 % 'Ion agar' was finally decided upon, as 4 % agar was too thick to dispense at 55–60° C. when egg-yolk emulsion was present. It was also found that proteus and other swarming organisms did not occur often enough to be a problem. The supplemented agar was prepared by adding to 100 ml. of agar base 1·5 ml. neomycin (10,000 μg./ml.) (Upjohn), 6·5 ml. Fildes extract (Oxoid), and 10 % egg-yolk emulsion (Oxoid). Ten per cent egg-yolk emulsion was decided upon as this produced a better Nagler reaction than either 5 % or 1 %, although these were fairly satisfactory.

This medium was made both with and without neomycin. This was because it was known (Dr E. S. Broughton, personal communication) that a heat-treated suspension of *Cl. welchii* (i.e. only spores) did not grow well on neomycin medium. However, it appeared that neomycin did not significantly influence the counts of the small number of *Cl. welchii* obtained. The results obtained from plates with neomycin were pooled with the counts from plates not containing neomycin.

Bacteriological analysis of surfaces

The method used was that of swabbing with alginate swabs (Higgins, 1950). Using a masking plate, a 2 in. diameter circle was swabbed three times. This method was used in preference to others, e.g. impression plate, because of a layer of dust at many of the sampling points. The three swabs were dissolved in 30 ml. of fluid and 0·5 ml. samples plated out on each of two aerobic plates and two anaerobic plates (one with neomycin, the other without).

Contamination of the humidifier and air sampling

Bacillus subtilis var. *globigii* was used. This organism produced a yellow-orange colony which could be easily identified. The bacteria were cultured in 16 oz. medical flat bottles containing 100 ml. of 2·5% peptone water and 0·5 ml. of Tween 80. About 40 bottles were used and these were laid on their sides. The Tween 80 was added to prevent pellicle formation. The bacteria were incubated at 37° C. for 7 days, centrifuged and resuspended in water. The centrifugation was necessary because humidifiers foamed with the broth and Tween, and the foam set free gave erroneous results. The bacteria were added to the humidifier in various dilutions and the water sampled for *B. subtilis* var. *globigii*. Three samples were taken and two sets of plates used for each. The air was sampled using the same method as for the filters but with Andersen samplers after the humidifiers and the secondary filters.

All bacterial samples throughout the experiments were incubated at 37° C. and counted after 36 hr.

RESULTS

Humidifiers

The number of bacteria given off by recirculatory type humidifiers

The concentration of the test bacteria, *B. subtilis* var. *globigii*, in the air after the humidifier, as compared to the concentration in the humidifier reservoir, is given in Table 4. The air sample was limited to 15 cu.ft. to avoid overdrying of the agar. Because of this it was not practicable to take tests with lower concen-

Table 4. *Concentration of* Bacillus subtilis *var.* globigii *emitted from two humidifiers compared with the amount in the reservoir*

(One test is a 15 cu. ft. sample of air,)

Humidifier type	No. of tests	Bacterial count/ml. water in reservoir	No. of bacteria per cu.ft. of air
Spray type	6	$3{\cdot}0 \times 10^3$	0·189
	4	$5{\cdot}2 \times 10^3$	0·213
	10	$9{\cdot}0 \times 10^3$	0·62
	6	$8{\cdot}6 \times 10^4$	1·335
	12	$2{\cdot}4 \times 10^5$	3·32
Capillary washer	6	$2{\cdot}5 \times 10^3$	0·100
	6	$3{\cdot}0 \times 10^3$	0·133
	8	$5{\cdot}8 \times 10^4$	0·732
	6	$3{\cdot}5 \times 10^4$	0·80

trations than those shown. These results were plotted on a graph and because of the similarity of results between the two humidifiers it was decided to combine the results as one. This similarity in results is probably because of the similarity of the design features which would be expected to influence the number of bacteria emitted, i.e. elimination plates, spray nozzles and water supply rate.

The results as in Table 4 were analysed to establish the relation between the two variables. By means of regression analysis the relationship was derived in two ways. First, the line of best fit was established. This is:

$$y = 0{\cdot}0000129x + 0{\cdot}213,$$

where y is the concentration of bacteria emitted from the humidifier per cu.ft. of air after the humidifier and x is the concentration of bacteria per ml. of water in the reservoir of the humidifier. The correlation coefficient is 0·99. Since it must be assumed that the line passes through the origin, i.e. when there are no bacteria in the humidifier reservoir no bacteria are given off, the data were recalculated. The equation for the line passing through the origin is

$$y = 0{\cdot}000014x$$

and the correlation coefficient is 0·989.

This line, which has a high correlation coefficient, is regarded as the relation between bacterial concentration in the reservoir of the humidifier and the number of bacteria given off into the air.

Size of particles emitted from the humidifiers

From the number of *B. subtilis* var. *globigii* obtained on the Andersen sampler stages it was calculated in the case of the spray washer that the equivalent median diameter was 2·2 μ. This was calculated from data of 16 tests, i.e. 240 cu.ft. of air sampled. Similarly, it was found from 14 tests that the equivalent median diameter of the bacteria being emitted from the capillary washer was 1·7 μ.

The effect of filtration by Vokes Univee Grade 'C' filters on bacteria given off by the humidifiers

On sampling a total of 210 cu.ft. of air before and after the secondary filter in the plant it was found that of the 162 *B. subtilis* var. *globigii* given off by the humidifier 71 bacteria passed through the filter. This is a removal efficiency of 56%.

It was also found that on testing the air in the area supplied by the plant, 4 hr. after the bacteria had been added to the humidifier water, a count of 0·5 bacteria/cu.ft. was obtained with four 15 min. tests. This compared to a figure of 2·8 bacteria/cu.ft. leaving the humidifier. The bacterial content of the water at that time was $2{\cdot}4 \times 10^5$/ml.

Concentration of bacteria in the humidifier reservoirs under normal conditions

Samples of the humidifier reservoir water were taken periodically over a year. With one exception, the concentration of bacteria in the samples taken never exceeded 40 bacteria/ml. The mean concentration was 21 bacteria/ml. The two

humidifiers usually had a constant overflow of water to dilute the bacteria and dust washed out of the air, but on one occasion the overflow stopped working. This was for a period of at least 4 weeks and on this occasion the bacterial concentration rose to $2{\cdot}05 \times 10^3$ bacteria/ml. before steps were taken to remedy the fault. These bacteria which were in almost pure culture were shown to be small Gram-positive rods probably *Corynebacterium* species and not thought to be potential pathogens.

Bacterial contamination of duct surfaces

Only Plant 1 was studied which had as a secondary filter a Univee Grade 'C' for full fresh air and a Univee Grade 'A' for recirculation. The results are considered in three groups. These consist of:

(*a*) The number of bacteria on the ducting from, and including, the extract grilles in the ward area to either the outside environment or the point of mixing with the fresh air before the primary filter. These are known as the recirculatory ducts.

(*b*) The number of bacteria on the supply ducts when full fresh air was being supplied.

(*c*) The number of bacteria on the supply ducts when two-thirds of the air was being recirculated.

The results are presented as total bacterial counts, *Cl. welchii* counts and *Staph. aureus* counts or, in the case of the recirculatory ducts, presumptive *Staph. aureus* counts.

Recirculatory ducts

Thirteen sampling points were chosen, four were at extract grilles, the remaining nine being positioned throughout the recirculatory duct system up to the point in front of the primary filter where the mixing of the fresh and recirculatory air took place. Approximately ten samples were taken at each position, 139 samples in all. The mean total count of bacteria was $1{\cdot}7 \times 10^3$/sq.in. of duct surface. This varied from $3{\cdot}4 \times 10^3$ bacteria/sq.in., the count at the extract grilles, to $1{\cdot}54 \times 10^2$ bacteria/sq.in. at the point before the secondary filter. This tendency to higher counts at the beginning of the recirculatory system falling by a 10 times reduction at the end of the system was reflected throughout the system—the further one went through the duct system the lower the count became. This was probably caused by two factors. It would be expected, because of impaction, that the concentration of particles would decrease down the duct system. It would also be expected in the larger ducts, apart from the lower concentration in the air, that the lower velocity should lead to less impaction. Anaerobic counts of *Cl. welchii* gave a mean count of 25·3 bacteria/sq.in., the range being from 0 to 193 bacteria/sq.in. Presumptive *Staph. aureus* counts accounted for approximately $\frac{1}{100}$ of the total bacterial counts, irrespective of the position at which the sample was taken.

Supply ducts with 100 % *fresh air*

Samples were taken before the primary filter and at various places throughout the supply system. A mean total count of $3{\cdot}66 \times 10^2$ bacteria/sq.in. was obtained before the primary filter. No *Staph. aureus* were isolated and the mean count of *Cl. welchii* was 9·5/sq.in.

The average count throughout the supply side, i.e. after the secondary filter, was 20 bacteria/sq.in. No *Staph. aureus* were isolated and out of 31 tests two *Cl. welchii* were isolated.

Supply ducts with two-thirds recirculation

Samples, taken at the same locations as used for the supply ducts with 100 % fresh air, showed an almost identical pattern. A total bacterial count of $1{\cdot}54 \times 10^2$/sq.in. was obtained before the primary filter, but a few *Staph. aureus* and *Cl. welchii* could be isolated. The *Cl. welchii* count was 20/sq.in. After the secondary filter the total bacterial count was 10/sq.in. Only two *Cl. welchii* and one *Staph.*

Table 5. *Air sampling throughout the air-conditioning plants*

		Air-conditioning plant					
		Plant 1 (ward area)			Plant 2 (central core)		Plant 3 (intensive care)
Amount of fresh air (%)		100	33 (C)*	33 (A)†	100	75	100
No. of tests		60	60	60	40	30	40
Intake	B	0·42	2·16	1·93	0·485	0·873	0·985
	B+A+M	1·71	3·14	2·08	2·51	3·21	4·21
After primary filter	B	0·071 (16·9)	0·557 (25·8)	0·424 (22·0)	0·064 (13·1)	0·146 (16·7)	0·152 (15·4)
	B+A+M	0·563 (32·9)	1·000 (31·8)	0·885 (42·5)	0·845 (33·7)	1·450 (45·2)	2·800 (66·5)
After humidifier	B	N.D.	N.D.	N.D.	0·061 (12·5)	0·091 (10·4)	0·096 (11·1)
	B+A+M	N.D.	N.D.	N.D.	1·050 (41·8)	0·854 (26·6)	1·665 (39·5)
Before secondary filter	B	0·035 (8·3)	0·318 (14·7)	0·183 (9·5)	0·061 (12·5)	0·121 (13·9)	0·105 (10·3)
	B+A+M	0·320 (18·7)	0·700 (22·3)	0·264 (12·7)	0·970 (38·7)	0·994 (31·0)	1·615 (38·4)
After secondary filter	B	0·035 (8·2)	0·198 (9·2)	0·013 (0·7)	0·046 (9·5)	0·079 (9·0)	0·091 (9·2)
	B+A+M	0·237 (13·9)	0·494 (15·7)	0·021 (1·0)	0·790 (31·5)	0·972 (30·3)	1·290 (30·6)

Mean bacteria counts (B) and mean micro-organism counts (B + A + M) are expressed as numbers per cu.ft. Figures in parentheses indicate percentage penetration. One test was 15 min. or 15 cu.ft. of air. N.D. indicates not done.

* Final filtration through grade C filter.

† Final filtration through grade A filter.

aureus were obtained out of 31 tests. More attention was paid to the supply diffusers and grilles, as evidence had been obtained suggesting a build-up of micro-organisms there. A mean bacterial count of 127/sq.in. and a mean *Cl. welchii* count of 10/sq.in. was obtained from 12 samples.

Penetration of the air-conditioning plants by bacteria and other micro-organisms

Total counts

The results obtained by air sampling at the points of the air-conditioning plants shown in Fig. 1 are given in Table 5. The counts per cu.ft. of bacteria (total bacterial count) and bacteria together with actinomycetes and moulds (total micro-organism count) are given. These counts are expressed also as the percentage penetration of the organisms with respect to the initial concentration.

The air samples were made up at times with large numbers of actinomycetes, which were morphologically distinct and could be easily picked out by their high phosphatase activity and characteristic colonial appearance. These actinomycetes were found mainly on stage 5 of the Andersen sampler and had an equivalent median diameter of 1·5 μ. These organisms arrived at various concentrations and at various times; concentrations as high as 14/cu.ft. were obtained when the total bacterial count was 1·8/cu.ft. Moulds, although not so erratic in their appearance in the air, had also a small equivalent median diameter (3·5 μ). The total micro-organism counts shown in Table 5 have, therefore, an erratic nature, and owing to the nature of the size distribution the air-conditioning systems were much less effective in removing them. Approximately 30 % of the total micro-organism count could pass through the air-conditioning system compared to approximately 9 % of bacteria.

The sampling point after the humidifier and before the secondary filter should give similar results, as the only obstruction between these points is a silencer. The results show reasonable similarity both with total micro-organism counts and total bacterial count which reflects well on the experimental reliability. In the case of plant 1, sampling after the humidifier was not possible owing to the inaccessibility of this part of the plant.

Staphylococcus aureus *counts*

These bacteria were not found to any large extent in the air. The mean count in the fresh air was 0·008/cu.ft. and in the recirculated air before the primary filter was 0·04/cu.ft. Occasionally throughout the three plants *Staph. aureus* could be isolated under all ventilation schemes, but few *Staph. aureus* were isolated after the secondary filter in any of the plants. In plants 1 and 3, under full fresh air, no *Staph. aureus* were isolated after the secondary filter but in plant 2 two *Staph. aureus* were isolated. Under recirculation in plant 3 two *Staph. aureus* were isolated whereas in plant 1 filtration through a Grade 'C' filter gave three *Staph. aureus* and filtration through Grade 'A' one. These *Staph. aureus* were the total number found throughout the tests. These are in line with the level which could be expected considering the initial concentration before the plants and the filtration efficiency of the plant.

Clostridium welchii *counts*

Tests were only carried out in plant 1 before the air-conditioning plant and after the secondary filter under two-thirds recirculation and full fresh-air ventilation. Fifty tests of 15 min. were carried out in each case. The concentration before the plant was 0·017/cu.ft. both with full fresh air and with two-thirds recirculated air. No *Cl. welchii* were found after the secondary filter.

Contamination of a secondary filter

In plant 1 it was found that the secondary filters had been overgrown with mould. The filter bags were wet to touch and it was apparent that free water was passing from the humidifier, which was just in front of the secondary filter, to the filter bags. Three 4 in. samples of the material (Vokes Univee Grade 'C') were homogenized and serial dilutions taken of the homogenate on to two plates each of Sabouraud dextrose agar (Oxoid), Oxoid blood agar base no. 2, and *Cl. welchii* medium. The mean counts obtained per sq.in. of material were:

Moulds	$2{\cdot}1 \times 10^5$	Aerobic bacteria	$1{\cdot}1 \times 10^2$
Yeasts	$1{\cdot}0 \times 10^6$	*Cl. welchii*	$1{\cdot}5 \times 10^2$

Although no identification was made of the aerobic bacteria isolated it could be seen from colonial appearance that a large percentage of them belonged to *Bacillus* spp.

Comparison of the size distribution of micro-organisms prior to the air-conditioning plant under full fresh air and two-thirds recirculation

This comparison was made to see if the air-conditioning plants would be subject to a different distribution of micro-organisms from the outside or the recirculated air, which would reflect on the filtration required.

The counts obtained from the individual stages of the Andersen samplers sited at the intake of plants 1, 2 and 3 under full fresh air were analysed to give the size distributions of the micro-organisms in the fresh air. The size distributions of micro-organisms in recirculated air were obtained for plant 1 only. Plant 2 was not tested, since only a quarter of the inside air was recirculated.

Statistical analysis

In order to assess the possibility that the size distributions were different for fresh air and recirculation the χ^2 test was applied. This was done by means of contingency tables and carried out in the case of bacteria, actinomycetes, moulds and *Staph. aureus*. The results are shown in Table 6. From these results, and the use of χ^2 tables, it can be seen that an obvious difference exists in the size distribution of bacteria, a small difference with the actinomycetes and moulds and little or no difference in the *Staph. aureus* size distributions. It must be realized in the case of *Staph. aureus* that the sample, especially with full fresh air, was very small.

Graphical analysis

By cumulative addition and plotting against 50% cut-off values of each Andersen sampler stage the results shown in Table 7 were obtained, as a comparison between full fresh air ventilation in plants, 1, 2 and 3 and recirculation in plant 1. Table 7 gives the equivalent median diameter and the geometric standard deviation of the size distributions of bacteria, *Staph. aureus*, moulds, actinomycetes and the total micro-organisms count, under full fresh air and recirculation.

Table 6. χ^2 *values for the difference in size distribution of micro-organism particles under full fresh air and recirculation*

Bacteria	197·60
Actinomycetes	17·37
Moulds	13·51
Staph. aureus	1·91

Table 7. *Equivalent median diameter (EMD) and geometric standard deviation (GSD), in microns, of micro-organisms in fresh air and recirculated air*

	Bacteria		*Staph. aureus*		Moulds		Actino-mycetes		Total micro-organism count	
	EMD	GSD	EMD	GSD	EMD	GSD	EMD	GSD	EMD	GSD
Full fresh air	16·0	5·3	9·2	4·9	3·6	2·0	1·7	2·3	2·8	1·9
Recirculation	6·4	3·3	8·9	4·2	3·5	1·9	1·6	2·0	4·5	2·6

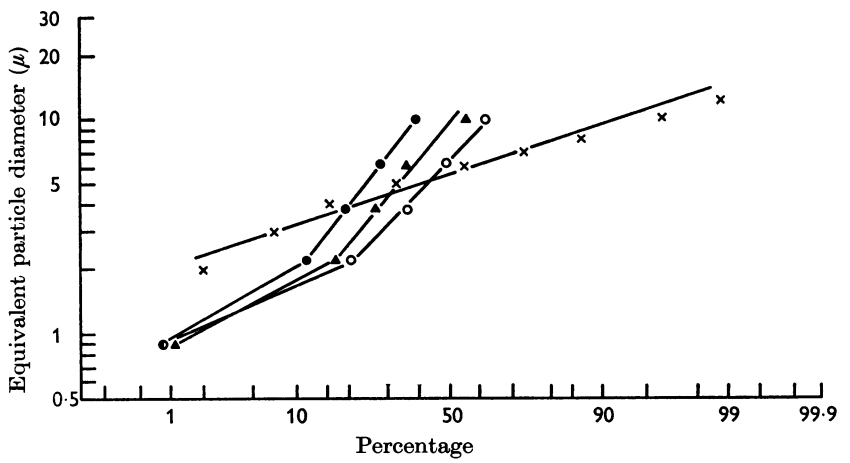

Fig. 2. Particle size distribution of *Staph. aureus* and bacteria under two-thirds recirculation and full fresh air. Also given is the size distribution by weight of Aloxite 50. ●, Bacteria full fresh air; ○, bacteria two-thirds recirculation; ▲, *Staph. aureus* under both fresh air and recirculation; × ——— ×, Aloxite 50.

A log-normal distribution was assumed and it was found that the results conformed very well to this distribution. It was only in the final stage of the sample that the results became truncated and, in the case of the moulds, stage 5. This one would expect at the tail of a log-normal distribution.

In Fig. 2 the size distribution of bacterial particles in fresh air and air of which two-thirds was recirculated are shown along with the size distribution of *Staph. aureus* particles in which size distribution in fresh air and recirculated air have been combined because of their similarity. Also shown is the size distribution by weight of Aloxite 50, a test dust conforming to B.S. 2831, Test Dust no. 2. In Fig. 3 the size distributions of moulds and actinomycetes are given.

It can be seen from this graphical method that under recirculation the medians of the sizes of all the micro-organisms are smaller except in the case of the total count. The differences are fairly small for moulds, actinomycetes and *Staph. aureus*, but the bacteria show quite a considerable difference in size. In the case of total micro-organisms, however, the median size in full fresh air is smaller than that of recirculation. This is because of the relatively larger numbers of larger bacteria present in the recirculated air, compared with the large numbers of smaller actinomycetes and moulds which are present in fresh air. This also shows the confusion that could arise if the actinomycetes had not been recognized and therefore treated separately.

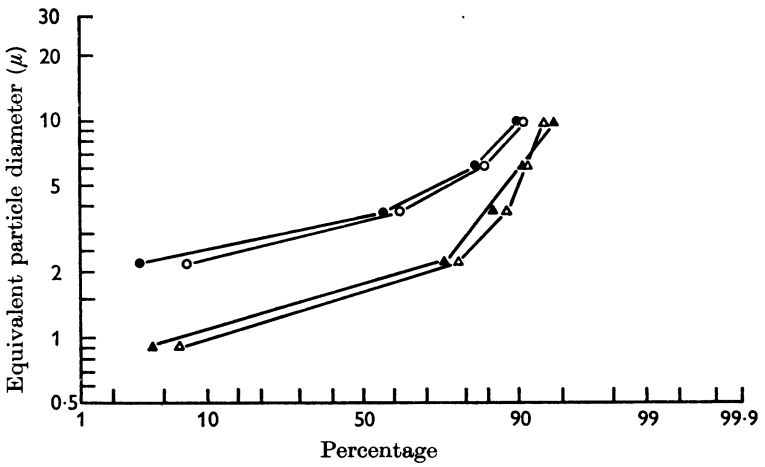

Fig. 3. Particle size distribution of actinomycetes and moulds under two-thirds recirculation and full fresh air. ▲, Actinomycetes full fresh air; △, actinomycetes two-thirds recirculation; ●, moulds full fresh air; ○, moulds two-thirds re-circulation.

DISCUSSION AND CONCLUSIONS

Humidification

It has been shown that by the artificial contamination of recirculatory storage tanks and humidifiers, bacterial concentrations can be obtained as given by the equation

$$y = 0{\cdot}000014x,$$

where y is the concentration of the bacteria particles given off by the humidifier per cu.ft. of air passing through the humidifier, and x the number of bacteria per ml. in the reservoir. This equation is only strictly applicable to the humidifiers tested, but it is unlikely that other humidifiers of these types would give results which differed markedly. Although these counts appear low it would not require

a high concentration of bacteria in the reservoir to give a concentration of bacteria in the air which would be comparable to the concentrations which were normally obtained in the air-conditioning system near the humidifier, i.e. counts of approximately 0·1 bacteria/cu.ft. The significance of the number of bacteria emitted from the humidifier is reinforced by the observation of the size and penetration power of the bacterial particles given off. The bacterial particles emitted from the humidifier are of droplet nuclei size, around 2 μ, and between 40 and 50 % of them are able to penetrate filters of filtering efficiency 91·8 % against B.S. 2831 Test Dust no. 2.

In normal circumstances the number of bacteria in the water used by the humidifier was less than 40 per ml., suggesting that the bacteria were not multiplying in the reservoir. This bacterial count was low enough to cause negligible trouble but this was in a well-maintained plant with a good overflow of fresh water through the recirculatory tank. In one case when the overflow was not functioning for a few weeks a count of $2{\cdot}05 \times 10^3$ bacteria/ml. was obtained. Although the bacteria found in this build up were not considered potential pathogens, for hospital purposes a safer method should be used, i.e. one which does not use recirculating water.

Duct surfaces

It has been shown that fairly large numbers of bacteria (an average of $1{\cdot}7 \times 10^3$ bacteria/sq.in. of duct surfaces), with a significant number of pathogens, are present on the extract side of the ventilation system, the numbers decreasing as one goes through the recirculatory system. It has been suggested that filters at each duct extract point should be provided or that regular cleaning should be given to the ducts to prevent these bacteria entering the room. No evidence was obtained to show that this was a significant problem but the design of the ventilation plant to avoid reverse flow should be considered in more critical areas where the ventilation plant may be shut off, e.g. operating theatres and treatment rooms.

The supply ducts of the air-conditioning plant had satisfactorily low counts of bacteria. Under recirculation the bacterial count was 10/sq.in. and under full fresh air 20/sq.in. Only the occasional pathogen was isolated. The exception to this satisfactory state of affairs was the inlet supply grilles and diffusers which showed a higher count of bacteria than the ducts supplying them. This was probably caused by impaction of entrained air from the room being supplied. The concentration of *Cl. welchii* on the inlet grilles and diffusers was significant enough to merit regular cleaning of the inlet points, especially in operating theatres and treatment rooms. The use of terminal filters does not appear to be justified as a bacteriological measure except where they are used instead of diffusers, as in laminar flow systems.

Filtration

The aim of these experiments was to produce data which could be used as design standards for the production of bacteriologically acceptable air in future hospital buildings. The bacteriological quality of air supplied by an air-conditioning plant, as far as filtration is concerned, is dependent on three points: the concentration and size distribution of the micro-organisms in the air to be filtered, and the efficiency of the filters against particles of these sizes.

The three types of microbiological airborne counts used in the evaluation of filters were the total number of aerobic bacteria (total count), the *Staph. aureus* count and the *Cl. welchii* count. Actinomycetes and moulds were excluded from a critical evaluation of the data because of their non-pathogenic nature, their unusually low equivalent median diameter (1·5 and 3·5 μ respectively) and their intermittent presence. Failure to recognize these actinomycetes could have given rise to results which would have underestimated the potential usefulness of filters. It is also possible that the higher counts of micro-organisms in the outside air obtained in some studies may be due to actinomycetes. In this study mean concentrations of 0·6 bacteria/cu.ft. and 2·4 micro-organisms/cu.ft. were obtained in the fresh air. The *Cl. welchii* count found in the outside air was 0·017 particles/cu.ft., whereas the *Staph. aureus* count was 0·008/cu.ft. These counts are in the region of those reported by previous authors, e.g. Blowers & Crew (1960), Lowbury & Lilly (1958), and Report (1948). In the case of recirculated air the bacterial concentration was 2·05/cu.ft., all micro-organisms 2·61/cu.ft., *Cl. welchii* 0·017/cu.ft. and *Staph. aureus* 0·04/cu.ft.

From the plotted distribution of the bacteria given in Fig. 2 the median diameter of bacterial particles in the recirculated air is 6·4 μ, those in the fresh air 16 μ. One of the limitations of the Andersen sampler for normal bacterial sampling is shown in Fig. 2 since only the size distribution of the smaller 50 % of the bacterial sample was obtainable for plotting the distributions. Some loss of larger particles would also be expected on the top sieve of the sampler (May, 1964). In the case of moulds and actinomycetes and also bacterial particles sampled after the filters, almost full size distributions were obtained. These particles were found on the lower stages of the sampler. The number of these particles lost would therefore be extremely small.

It can be seen that the size distributions of bacteria under full fresh air and recirculation converge; the difference between the two diminishing as the size of the bacteria decreases. One would therefore expect that primary filtration, by removing the larger bacterial particles, would tend to reduce the differences between the size distributions of the bacteria. In Table 5 this is substantiated in that 16 % of the bacterial particles in the fresh air penetrate the primary filters in plants 1 and 3, compared with approximately 24 % of the bacterial particles in the recirculated air. This difference is not in itself very great but it can be seen that under both recirculation and full fresh air the penetration of the secondary filters by the bacterial particles is approximately 9 %. In the case of filtration in plant 2 where the recirculated volume is only one-quarter of the total volume, the

figures for penetration through the primary filter are fairly similar, 13·1 and 16·7 %. At the secondary filters the similarity is even more striking with 9·5 and 9·05 % penetration. This evidence suggests that recirculated air and fresh air have size distributions which are similar enough for primary filtration to reduce the difference to practical insignificance. However, owing to the presence of greater numbers of *Staph. aureus* higher standards of filtration are probably advisable.

The three air-conditioning plants supplied air with concentrations of total bacteria and pathogenic bacteria which appeared quite satisfactory. It was found that passing fresh air through an air-conditioning plant which had a final filtration efficiency of 91·8 % to B.S. 2831 Test Dust no. 2 gave a count of 0·035–0·09 bacteria/cu.ft. On recirculation of two-thirds of the ward air and a secondary filter of efficiency of 99·35 % to B.S. 2831 Test Dust no. 2 a mean concentration of 0·013 bacteria/cu.ft. was obtained. Even better removal efficiency than the 99·33 % removal obtained through this filter was probable since in the 60 tests only 12 bacteria were found which had apparently penetrated the filter. The possibility that several of these bacteria were inadvertently introduced into the 360 Petri dishes used seems a real one.

Shown in Fig. 2 along with the size distribution of bacteria and *Staph. aureus* is the size distribution of Aloxite 50, the test dust used in testing filters to B.S. 2831 Test Dust no. 2. The size distribution of this test dust approximates nearest of any of the test dusts to the size distribution of the bacteria and *Staph. aureus*, although it is slightly smaller. It can be seen in Table 5 that the removal efficiency of the primary filters against bacteria is less efficient than against B.S. Test Dust no. 2. If, however, the results of penetration of the secondary filters are considered it can be seen that a fair correlation between the efficiencies is obtained. This is shown in Table 8.

Table 8. *Comparison of the removal efficiencies of the secondary filters against bacteria and Aloxite* 50

Type of filter	Percentage efficiency against bacteria	Percentage efficiency against Aloxite 50
Univee Grade 'A'	99·3	99·35
Univee Grade 'C'	90·8, 91·8, 90·8	91·8
Miniroll Standard	90·5, 91·0	87·5

Similarly, results taken from the work of Blowers & Crew (1960) show that filtration of fresh air through a K 600 Kompak rated 97·4 % efficient to Aloxite 50 was 97·85 % efficient in the removal of bacterial particles. It would seem, therefore, that a fair approximation to the filtering efficiency of a filter to bacteria in the air can be obtained by reference to the filter's quoted efficiency to Aloxite 50; the bacterial removal efficiency being equal to the quoted efficiency. This approximation is dependent on the fairly standard practice of a filter of efficiency approximately 90 % to Aloxite 50 being used as a primary filter.

From the results given it is felt that a filter can be chosen for the purpose in hand. Although standards may vary from situation to situation depending on

several variables, the author would give the following standards for guidance. Primary or pre-final filtration is assumed to be approximately 90% to B.S. 2831 Test Dust no. 2.

	Fresh air %	Recirculated %
Non-critical areas, e.g. general wards, corridors, etc.	90	99
Critical areas, e.g. operating theatres, treatment rooms, isolation rooms, etc.	99	>99

The percentage figures are bacterial removal efficiency figures or the quoted efficiency of the filter to Aloxite 50.

It was found, in plant 1 only, that moulds and yeast but no bacteria were growing in the secondary filter. This was because of the close proximity of the humidifier to the filter. This could be overcome by either moving the filter away from the humidifier, using more effective means of eliminating the water carried over from the humidifier, or by placing the heater batteries in front of the secondary filter (this would be impractical with multizone reheat).

No experimental observations were made of the ability of viral particles to pass through filters, and no work on this topic appears to have been published. It is probable that viral particles are not of viral size but are released from humans accompanied by body materials; it is also known that some viruses lose their viability quickly (Tyrrell, 1967). It seems possible, therefore, that normal standards of filtration may suffice. Owing to lack of sources and the large amount of dilution which will occur if viruses are dispsersed into the outside air, it is doubtful if fresh air contains many viruses. Until more information is made available it is suggested that in areas where viral infection is considered a problem ventilation should be by fresh air, or the area concerned be regarded as 'critical' for the purposes of filtration of recirculated air.

From the data given it is felt that the air-conditioning plants in the experimental ward did all that was required of them in producing air for use in the ward area and it is hoped that the results obtained can be used in other hospital situations.

SUMMARY

An investigation was carried out into the bacteriological performance of three air-conditioning plants in a hospital ward. Two of these plants had the facility for recirculating part of the ward air.

An equation has been derived comparing the concentration of bacteria which would be expected to be given off by the humidifiers in the ventilation system, with the concentration of bacteria in the recirculatory tank. The bacterial particles given off by these humidifiers were of nuclei droplet size, and were found to penetrate the filters used with a fair degree of ease. Although the number of bacteria in the humidifier water remained insignificant with a constant overflow of water into the recirculatory tank, on one occasion a build-up of bacteria was demonstrated when the overflow ceased. For hospital use humidifiers of a non-recirculatory type should be used.

The concentration of bacteria on the surface of the recirculatory ducts was

assessed, as also were those on the surface of the supply ducts under full fresh air and recirculation. The concentration of bacteria in the supply ducts was low and the use of terminal filters was not merited, although care should be taken to prevent the build-up of bacteria in inlet grills and diffusers. The bacterial concentration in the exhaust ducts was found to be quite high. It was therefore thought that in critical areas, where the ventilation plant may be shut off, the use of some device to prevent reversed air flow may be necessary.

The count of various types of micro-organisms in the fresh air and two-thirds recirculated air are given along with their size distribution. The results of the effect of filtration on the concentration of bacterial particles throughout the air-conditioning plant is given under full fresh air and recirculation. These concentrations appear quite satisfactory. It was found that one set of filters had been overgrown by mould because of free water being brought over from the humidifier. Measures have been suggested to overcome this. When primary or prefinal filtration was approximately 90 % efficient to Aloxite 50 (B.S. 2831 Test Dust no. 2) it was demonstrated that a fair approximation to the final filtration figure could be obtained by reference to the quoted efficiency of the final filter to Aloxite 50. After similar primary filtration it was demonstrated that the final filtration of filters against recirculated and fresh air was approximately the same. Owing to the higher number of *Staph. aureus* in recirculated air, higher efficiency filtration may be required.

Standards of filtration efficiency for critical and non-critical zones are suggested.

The author would like to thank Mr R. Rutherford and Mrs M. Marshall for technical assistance, Mr W. Carson and members of the Building Services Research Unit for advice and guidance, and Sister M. Muir and the staff of the Hairmyres Experimental Ward for their support and help. This work was financed by the Nuffield Provincial Hospital Trust.

REFERENCES

Andersen, A. A. (1958). New sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bact.* **76**, 471.

Barber, M. & Kuper, S. W. A. (1951). Identification of *Staphylococcus pyogenes* by the phosphatase reaction. *J. Path. Bact.* **63**, 65.

Blowers, R. & Crew, B. (1960). Ventilation of operating theatres. *J. Hyg., Camb.* **58**, 427.

Blowers, R., Lidwell, O. M. & Williams, R. E. O. (1962). Infection in operating theatres in relation to air conditioning equipment. *J. Instn Heat. Vent. Engrs* **30**, 244.

Blowers, R., Mason, G. A., Wallace, K. R. & Walton, M. (1955). Control of wound infection in a thoracic surgery unit. *Lancet* ii, 786.

Higgins, M. (1950). A comparison of the recovery rate of organisms from cotton-wool and calcium alginate wool swabs. *Mon. Bull. Minist. Hlth* **9**, 50.

Kethley, T. W., Cown, W. B. & Fincher, E. L. (1962). Bacterial load of air in simulated operating rooms. *Progress Report OH-19. Engineering Experiment Station, Georgia Institute of Technology, Atlanta.*

Lowbury, E. J. L. (1954). Air conditioning with filtered air for dressing burns. *Lancet* i, 292.

Lowbury, E. J. L. & Lilly, H. A. (1955). A selective plate medium for *Clostridium welchii*. *J. Path. Bact.* **70**, 105.

Lowbury, E. J. L. & Lilly, H. A. (1958). The sources of hospital infection of wounds with *Clostridium welchii*. *J. Hyg., Camb.* **56**, 169.

MAY, K. R. (1964). Calibration of a modified Andersen bacterial aerosol sampler. *Appl. Microbiol.* **12**, 37.

MULCASTER, K. D. & STOKES, E. A. (1966). The aspect of filtration in ventilation and air conditioning systems. *J. Instn Heat. Vent. Engrs* **34**, 197.

REPORT (1948). Studies in air hygiene. *Spec. Rep. Ser. med. Res. Coun.* no. 262.

REPORT (1962). Design and ventilation of operating-room suites for control of infection and for comfort. *Lancet* ii, 945.

REPORT (1964). The Hairmyres Experimental Ward Unit. *J. Instn Heat. Vent. Engrs* **32**, 330.

SHAFFER, J. G. (1963). The laboratory in infection control. National Conference on Institutionally Acquired Infection. September, 1963. *Publ. Hlth Serv. Publs, Wash.* no. 1188.

SHOOTER, R. A., TAYLOR, G. W., ELLIS, G. & ROSS, J. P. (1956). Postoperative wound infection. *Surgery Gynec. Obstet.* **103**, 257.

TYRRELL, D. A. J. (1967). The spread of viruses of the respiratory tract by the airborne route. In *Airborne Microbes. Symp. Soc. gen. Microbiol.* no. 7.