THE USE OF RADIATION TO ESTIMATE THE NUMBERS OF MICRO-ORGANISMS IN AIR-BORNE PARTICLES

By O. M. LIDWELL and W. C. NOBLE
Air Hygiene Laboratory, Central Public Health Laboratory, Colindale, N.W. 9
and G. W. DOLPHIN
Physics Department, St Bartholomew's Hospital Medical College, Charterhouse Square, London, E.C. 1

(With 1 Figure in the Text)

INTRODUCTION

The realization that cross-infection by strains of *Staphylococcus aureus* is a frequent experience within a hospital has stimulated a number of investigations (a summary is given by Williams, 1959). The use of phage typing has shown that the strains of staphylococci present in any one situation, e.g. a ward, are usually continually changing and that most strains which appear are found in only relatively small numbers and disappear again without any clinical consequences. From time to time, however, strains appear which are found in much larger numbers and some of these exhibit a considerable facility for colonizing particular sites, e.g. the noses of patients and nurses. A small minority of strains produce disease, sometimes manifested in the form of widespread and persistent epidemics. Certain phage types appear to be associated with this disease-producing potentiality but the association is by no means a complete one. Any other ways in which the disease-producing strains could be shown to differ from the others would clearly be of considerable interest. One possible difference is variation in the numbers of viable cocci carried by the air-borne bacteria-carrying particles, which may be the vector of infection. If there were any considerable difference in this respect, then estimates of the potential risk of infection, based on the numbers of viable particles in the environmental air, would be misleading quite apart from any variation in actual invasiveness of the strains. Larger numbers of viable cocci in the air-borne particles could arise in a variety of ways, including: growth to a higher density in the menstruum from which dispersion is taking place, better survival of cocci during and following the drying of the dispersed material, physical changes in the infected menstruum affecting the way in which it breaks up on dispersion, or greater adhesion between the individual cocci.

When air-borne particles are sampled on to a solid medium in a slit sampler, each particle gives rise to a single colony irrespective of the number of viable units it may be carrying. By contrast, sampling into a liquid medium, followed by plating out of the whole or part of the fluid, might be supposed to break up any bacterial aggregates, partially or completely, and give rise to a higher count than that obtained by sampling directly on to the solid medium. The difference between the counts obtained by these two methods would then give an estimate of the state

of aggregation of the original material. Some early experiments on samples of the naturally occurring flora of occupied places did not reveal any significant difference in the counts obtained by the two methods. Comparisons of this kind, however, are essentially indeterminate, since they can only be interpreted as indicating a minimum value of the degree of aggregation. If the two methods show no difference it may be that the air-borne particles are carrying only single cocci; it is, however, possible that the aggregates are too firmly bound together to be separated by means which do not themselves endanger the viability of the individual organisms.

A completely different approach is possible. If the particles are exposed to the action of some lethal agent which acts independently and equally on each individual coccus so that the chance of death is identical for each infinitesimal increment of dose, then the chance of survival of any coccus following a dose d is given by

$$P = q^d, \tag{1}$$

where q is a constant less than one.

[This is identical with the common exponential decay since

$$\ln P = d \ln q$$

and $$P = e^{-ad}, \quad \text{where} \quad a = -\ln q.]$$

The chance of one or more survivors from a clump of n cocci is then given by:

$$\begin{aligned} P_n &= 1-(1-q^d)^n \\ &= nq^d - \frac{n.\overline{n-1}}{2!}q^{2d} + \frac{n.\overline{n-1}.\overline{n-2}}{3!}q^{3d}\ldots. \end{aligned} \tag{2}$$

As d increases $$q^d \to 0,$$

$$P_n \to nq^d,$$

and $$\log P_n \to \log n + d\log q, \tag{3}$$

i.e. the relationship between $\log P_n$ and d tends to a straight line which intersects the $\log P_n$ axis at a value of $\log P_n = \log n$.

If there is a distribution of clump size such that f_n = the relative frequency of clumps containing n individual cocci,

then $$\Sigma n f_n = N \quad \text{and} \quad \frac{\Sigma n f_n}{\Sigma f_n} = \bar{n},$$

where N is the total number of units and $\bar{n}$ the arithmetic mean clump size. The fraction of aggregates surviving a dose d is now given by:

$$\begin{aligned} P_{\bar{n}} &= \frac{1}{\Sigma f_n}\Sigma\left(f_n\left[nq^d - \frac{n.\overline{n-1}}{2!}q^{2d}\ldots\right]\right) \\ &= \frac{1}{\Sigma f_n}(Nq^d - \tfrac{1}{2}[\Sigma n^2 f_n - \Sigma n f_n]q^{2d}\ldots) \\ &= \frac{1}{\Sigma f_n}(Nq^d - \tfrac{1}{2}[\Sigma(n-\bar{n})^2 f_n + 2\bar{n}\Sigma n f_n - \bar{n}^2\Sigma f_n - \Sigma n f_n]q^{2d}\ldots) \\ &= \bar{n}q^d - \tfrac{1}{2}(\sigma^2 + \bar{n}^2 - \bar{n})q^{2d}\ldots \end{aligned} \tag{4}$$

where σ is the standard deviation of n, the clump size. As d increases

$$P_{\bar{n}} \rightarrow \bar{n}q^d$$

and

$$\log P_{\bar{n}} \rightarrow \log \bar{n} + d \log q \tag{5}$$

and again the relationship between $\log P$ and d tends to linearity as d increases and the limiting straight line intersects the $\log P_{\bar{n}}$ axis at a value of $\log P_{\bar{n}} = \log \bar{n}$. It is of some interest to consider the rate at which the relationship between $\log P$ and dose approaches the limiting straight line.

For uniform clump size

$$P_n = nq^d\left(1 - \frac{n-1}{2}q^d\right) + \ldots \tag{6}$$

and the percentage deviation of P from the limiting line at a given value of d is approximately half the percentage of clumps surviving so long as this is small, e.g. $< 0{\cdot}1$. Where there is a distribution of clump size

$$P_{\bar{n}} = \bar{n}q^d\left(1 - \tfrac{1}{2}\left[\frac{\sigma^2}{\bar{n}} + \bar{n} - 1\right]q^d\right) + \ldots. \tag{7}$$

If the distribution is Poissonian with $\sigma^2 = \bar{n}$ then the deviation from the limiting line is only very slightly greater than for a uniform clump size. If the standard deviation of n is larger than this then the deviation from the limiting line increases.

While a variety of agents, chemical and physical, produce logarithmic or near logarithmic disinfection curves when organisms are uniformly exposed to them, most of them are not suitable for a sample of air-borne material, which is most conveniently obtained on the surface of the selected medium. With chemical agents there is considerable uncertainty as to whether the process is truly logarithmic over a wide enough range and there are also uncertainties introduced by the possibility of various barriers to diffusion of the agent. Ultra-violet radiation is too readily absorbed by organic matter, or screened by opaque objects to produce with certainty uniform exposure of each individual organism. X-rays fulfil the required criteria but examination of the dose required for kills exceeding 99·9 % shows that their use would not ordinarily be a practical proposition. Suitable dose rates of high-energy electrons can, however, be obtained from a linear accelerator and the work reported here has been carried out with this type of instrument.

A large proportion of the work on the sterilization of micro-organisms with high energy electrons has been carried out with a view to the preservation of foodstuffs, the sterilization of pharmaceuticals or for other practical ends. This has usually involved irradiation in complex media but there are a number of experimental observations reported in the literature which are relevant to this investigation. In particular, Moriarty (1950), working at the Massachusetts Institute of Technology, has irradiated suspensions of *Staph. aureus* in distilled water, in a series of gelatin solutions and in a nutrient broth. The dose required to obtain a tenfold reduction in the number of viable organisms increased from 16 krad. for the distilled water suspensions to 21 krad. in 0·001 % gelatin, 27·5 krad. in 0·1 % gelatin and 44 krad. in nutrient broth. In general the removal of oxygen and the introduction of

reducing agents increases the dose required for a given percentage sterilization. Various other micro-organisms were also examined. The vegetative forms required doses which can be estimated to range from 10 to 60 krad. for 90 % sterilization in nutrient broth suspensions, while the spores required doses of 150–250 krad. The linearity of the logarithmic killing curves has been more closely examined for X-rays than for electron bombardment, but where the point has been considered the linearity of the relationship has been confirmed. The mean value of linear energy transfer was 7·4 keV./μ for the 13 MeV. electrons used in this work. This differs only slightly from the value of about 10 keV./μ for 250 kV. X-rays. The biological effectiveness of the electron beam might, therefore, be expected to be similar to that of 250 kV. X-rays.

EXPERIMENTAL METHODS

The source of radiation used was the 15 MeV. linear accelerator at St Bartholomew's Hospital (Rotblat, 1955). In order to obtain a uniform dose rate over a large enough field, the electron beam, after being deflected vertically downwards, was scattered by means of a gold foil 0·66 mm. thick. With this arrangement the dose rate was constant within $\pm$ 5 % over a 15 cm. diameter circle at the working position. The radiation dose was monitored by integrating the ionization current from a Perspex ionization chamber placed at a fixed position in the beam. The chamber collection efficiency at the dose rate employed, about 40 krad./min., was approximately 95 % so that small changes in the dose rate did not cause uncertainty in the estimation of the dose. The ionization chamber was calibrated using Perspex dosimetry (Boag, Dolphin & Rotblat, 1958). The Perspex slips used for the calibrations were placed in the same positions as the organisms to be irradiated.

The samples for irradiation were placed in some or all of twelve stations on the periphery of a 5 ft. diameter horizontal disk (Lindop & Rotblat, 1958) which could be rotated from outside the radiation room so as to bring each station in turn into the electron beam.

For the preliminary experiments with broth cultures and freeze-dried preparations the samples were exposed after spreading on to the surface of an agar medium contained in 4 in. diameter glass Petri dishes, or in talc powder in plastic containers.

The samples of air-borne organisms were collected on the surface of agar media contained in 5½ in. diameter glass Petri dishes. In view of the inherently variable nature of the air-borne samples it was necessary to expose each plate to a series of doses. This was done by means of ½ in. thick lead shields which could be lifted from outside the radiation room so as to expose different sectors of the plate to varying fractions of the total dose. A 60° sector of the plate was protected throughout, the two 60° sectors lying on either side of this received differing fractional doses while the remaining half of the plate received the full dose. The small numbers of organisms in the individual samples, together with the errors of positioning the plates relative to the shields and the effects of scattering, made any finer subdivision of the plates unprofitable. The use of a bigger sector at the highest

dose level was mechanically convenient and at the same time it increased the very small number of survivors found at this level. Although this procedure gave estimates for only three points on the dose response curve, the two higher levels were chosen to approximate to the asymptotic linear portion of the curve so that reasonable estimates of the radiation sensitivity and clump size could be obtained.

In order to increase the number of plates which could be irradiated in a given time two plates were exposed one on top of the other in each station. They were held in position in aluminium holders, glass bottoms upward, with their aluminium lids in position. Tests showed that the dose received on the surfaces of the medium in the two plates of a pair was substantially identical.

RESULTS

Evaluation of the results

In the preliminary experiments the unirradiated controls were never taken into the radiation room and the whole of each test-plate received the same dose. It was possible, by plating out dilutions, to use very large numbers of organisms for the control samples so that the killing curve could be followed over a large range and the asymptotic portion of the curve closely approximated. Estimation of the radiation sensitivity and mean clump size could then be simply made by extrapolating this back to the zero dose axis, see equation (5) above. Evaluation of the results obtained with the samples of air-borne organisms was not quite so straightforward. Owing to the production of X-rays in the gold foil used for scattering, the electron beam contained a proportion of X-rays. These X-rays were only slightly absorbed by the lead shields while at the same time additional X-rays were produced as the electron beam was absorbed in the lead. As a result of this the X-ray dose reaching the plate was very little affected by the interposition of the shields and the whole surface of the plate received an X-ray dose which was approximately equal to 5 % of the total dose directed at the plate, i.e. if the full dose were 80 krad., 4 krad. was in the form of X-rays and this was received by the whole surface of the plate including the nominally unirradiated sector. A dose series which would have delivered doses of 0, 20, 40, 80 krad. to the four sectors if the lead shields had acted as perfect absorbers resulted, therefore, in the four sectors receiving doses of 4, 23, 42 and 80 krad., respectively. In addition, scattering of the electron beam in the plates and medium caused blurring of the sector boundaries. The extent of this blurring was estimated photometrically on photographic film exposed at the surface of the agar medium of the plates. From this data the dose distributions actually received by the different sectors of the plate were estimated. Sets of values of the fraction surviving on the different sectors were then calculated for these dose distributions for a series of sensitivities and clump sizes, using equation (2), and those values of sensitivity and clump sizes which gave the best approximation to the observed values derived by minimizing the sum of the squares of the differences between the logarithms of the observed and calculated values of the fractions surviving.

This method of correcting for the effects of scattering was tedious but produced

a considerable improvement in the correspondence between the observed killing curve and the theoretical form. The effects of scattering can be largely eliminated by excluding a $\frac{1}{4}$ in. wide strip on each side of the nominal sector boundary from the area on which colonies are counted, unfortunately the desirability of this was not realized until after the series of observations reported here had been completed.

Preliminary experiments with cultures

Broth cultures of two strains of *Staph. aureus* (NCTC 8319 and 8510) and of one strain of micrococcus (NCTC 7944) were diluted 1:10 in saline and homogenized

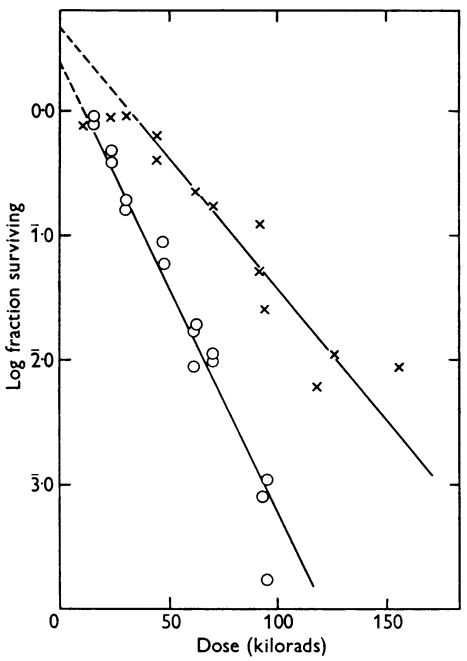

Fig. 1. Dose survival curves for suspensions of cocci on an agar surface. ○, two strains of *Staphylococcus aureus*; ×, a micrococcus.

by shaking with small glass ballotini in the Mickle disintegrator for 5 min. Microscopic examination of stained smears showed that even after this treatment the cocci were to a considerable extent still associated in pairs, triads and some larger groups. A series of tenfold dilutions of the homogenized suspensions were then made up in 10% broth saline and aliquot portions (1/10 ml.) of these plated out immediately before irradiation. The staphylococcal suspensions were plated on to a nutrient agar medium containing 5% horse serum and 0·01% phenolphthalein phosphate (Barber & Kuper, 1951), the micrococcus being plated on to a simple nutrient agar. Colonies were counted after 18–20 hr. incubation at 37° C. Control platings were also made on to plates which had previously been irradiated at the maximum dose. The maximum dose employed in the several series of experiments ranged up to 120 krad. for the staphylococcus and up to 183 krad. for the micrococcus. The results of these experiments are shown in Fig. 1; there was no

detectable differences between the different strains of staphylococci. The slope of the limiting line approached 27 krad. for a tenfold reduction in survivors, the curve was followed down to 0·01 % survivors, and the intercept on the zero dose axis indicated a mean clump size of 2·5 cocci. The micrococcus was more resistant to the radiation, the limiting line indicated that 47 krad. were necessary for a tenfold reduction in survivors while the mean clump size indicated by the intercept was 4·4. This curve was followed down to below 0·1 % survivors.

There was no indication of any inhibition of growth on the pre-irradiated control plates.

Two additional experiments were performed with one of the staphylococcal strains (NCTC 8319). In the first a set of plates seeded with aliquots from the dilution series as described above was incubated for 2½ hr. at 37° C. before irradiation. Some of these were then respread with glass rods to disperse the micro-

Table 1. *Irradiation of cultures*

Organism	Conditions	Sensitivity (krad./tenfold reduction in survivors)	Mean* clump size
Staph. aureus	Ground in dried talc	35	4·5
Staph. aureus	Homogenized culture spread on to agar surface	27	2·5
Staph. aureus	Micro-colonies on agar surface	(27)†	12
Micrococcus	Homogenized culture spread on to agar surface	47	4·4

* The mean number of viable units (cocci) in the bacterial aggregate.

† In estimating the mean clump size from the results of this experiment the sensitivity of the organisms was assumed to be the same as that found for the cultures in the preceding experiment.

colonies which had formed, the remainder being exposed to the electron beam. The plates respread after the 2½ hr. incubation, when incubated for a further 20–24 hr., showed a 4·4-fold increase in colony count over similarly inoculated plates which were not respread in this way. The dose survival curve for the irradiated plates indicated a mean clump size, in the micro-colonies, of 12 cocci, which is 4·8 times the mean clump size on the plates that had not been incubated. The close agreement between these two ratios confirm that the method can detect relatively small changes in mean clump size, if we can assume that the mean clump size of the respread cocci from the micro-colonies was similar to that in the original suspensions. In the second additional experiment freeze-dried cultures of this strain were ground up into dry sterile talc powder and portions exposed to irradiation in plastic containers. The contents of the containers was then suspended in broth saline, dilutions made and aliquot portions of these plated out. The dose survival curve gave a mean clump size of 4·5 and a sensitivity of 35 krad. for tenfold reduction in survivors.

The results of these experiments are given in Table 1.

Experiments with artificially produced air-borne clouds

Samples were taken with the size-grading sampler (Lidwell, 1959) on to plates containing S 1 medium (Williams & Hirch, 1950) from the air of a room into which salivary secretion had been finely dispersed by means of a spraying spit (Bourdillon, Lidwell & Lovelock, 1948). These plates were then exposed to the electron beam and colonies of *Streptococcus salivarius* counted after 36 hr. incubation at 37° C. The pooled results of three such experiments are given in Table 2. It will be seen that the streptococci are much more sensitive than the staphylococci to the effects of irradiation and that the indicated mean clump size increases regularly with particle size.

Table 2. *Irradiation of air-borne salivary particles*

Sampler stage	Approx. size range of air-borne particles*	Survivors after a nominal dose of 0 krad.	23 krad.	46 krad.	69 krad.	Mean clump size
A	$> 18\mu$	2,726	769	116	3·5† (8)	11·0
B	$10–18\mu$	6,110	1,590	186	7·2 (17)	8·9
C	$4–10\mu$	6,160	1,202	125	3·4 (8)	4·7
D	$< 4\mu$	4,465	414	62	0 (0)	2·4
All together		19,461	3,975	48·9	11·1 (33)	5·9
$-\log P$		—	0·69	1·60	3·24	—
$-\log P$ (calc.)		—	0·66	1·66	3·21	—

* The sizes given are 'equivalent particle diameters', i.e. the diameters of spherical particles of unit density which have the same settling rate in air.

† The fractional numbers are the results of correcting the actual sector areas exposed to this dose to the sector areas exposed at the other three dose levels. The actual numbers of colonies counted were as shown in brackets.

$\log P$ = log (survivors after indicated nominal dose/survivors after nominal dose 0). The calculated values are derived as described in the text for a mean clump size of 5·9 and a sensitivity of 16·7 krad. for a tenfold reduction in survivors.

Experiments with naturally occurring air-borne clouds

Samples were taken with the size-grading sampler from two hospital wards using the serum agar medium containing phenolphthalein-phosphate. The total sampling time over the whole fifteen visits exceeded 22 hr. and the total volume of air sampled was more than 27,000 cu.ft. The plates were exposed to the electron beam between 2 and 6 hr. after the samples had been collected. During this time they were kept at room temperature. Colonies of *Staph. aureus* and other organisms were counted after about 20 hr. incubation at 37° C. The nominal dose levels used were 23, 46 and 92 krad. together with the nominally unexposed control sector.

Since the air-borne flora is very heterogeneous, no great significance can be attached to the dose survival curve for all the colonies counted and this was only examined on a few occasions. Over the range explored there was a slight tendency for the curve to bend upwards with increasing dose as the action of the irradiation left only the more resistant strains surviving.

On two occasions appreciable numbers of spore-bearing organisms were

encountered. On one occasion these appeared to be *Bacillus subtilis*; those found on the other occasion were not identified. These strains showed the high resistance characteristic of spores, the dose required for a tenfold reduction of survivors appeared to exceed 200 krad. The mean particle size was much smaller than the average for the air-borne flora and it is possible that these organisms were largely present as individual spores.

Staph. aureus was found in samples from every ward visit but in very varying numbers. The numbers of colonies identified per cu.ft. of air sampled (averaged over a single visit) varied from 0·04 to 1·8. The actual numbers of colonies found in the samples are given in Table 3.

Table 3. Staphylococcus aureus *colonies in air samples*

Ward	Type	Sampler stage	Approx. size range of air-borne particles*	Survivors after a nominal dose of 0 krad.	23 krad.	46 krad.	92 krad.	Mean clump size
W	Mixed	All together		365	90	36	3/3†	3·7
H	187	All together		1368	490	160	7/3	5·2
H	53	All together		169	36	7	0	1·7
W	6/47/53	All together		664	183	47	0	3·1
All together		A	$> 18\,\mu$	845	320	110	2/3	6·0
		B	$10–18\,\mu$	1063	344	99	7/3	4·2
		C	$4–10\,\mu$	498	119	35	1/3	3·0
		D	$< 4\,\mu$	160	16	16	0	*ca.* 1·0
		All together		2566	799	250	10/3	4·0
		$-\log P$		—	0·51	1·01	2·89	—
		$-\log P$ (calc.)		—	0·44	1·10	2·87	—

* The sizes given are 'equivalent particle diameters', i.e. the diameters of spherical particles of unit density which have the same settling rate in air.

† The divisor 3 is introduced since the sector areas exposed at this dose were three times as large as those exposed to the other three levels.

$\log P$ = log (survivors after indicated nominal dose/survivors after nominal dose 0). The calculated values are derived as described in the text for a mean clump size of 4·0 and a sensitivity of 25·8 krad. for a tenfold reduction in survivors.

In ward W, the counts were uniformly low on the first ten visits, the average values lay between 0·04 and 0·15 colonies per cu.ft. of air, and the staphylococci were of very varied phage types; these results may then be considered as representative of the 'background' level. In ward H, the counts were considerably higher. On three occasions when the average counts were 1·8, 1·1 and 0·6 colonies per cu.ft. of air the *Staph. aureus* colonies identified were almost entirely of one phage type, 187. On the fourth visit the average count was 0·4 colonies per cu.ft. and the staphylococci were predominantly type 53. An eleventh visit to ward W produced an average count of 1·8 colonies of *Staph. aureus* per cu.ft., all those identified being of type 6/47/53. These results can therefore be considered as examples of active dissemination. There was, however, no evidence of the spread of disease due to any of these types.

The sensitivity to electron bombardment of the staphylococci recovered from the air, 26 krad. for a tenfold reduction in survivors, did not differ significantly

from the value, 27 krad., found for organisms from broth cultures irradiated on the surface of an agar culture medium. The mean clump size shows a small but regular increase with particle size. The apparent differences in the clump size associated with the different phage types are of doubtful significance in view of the size of the samples and the experimental errors involved. In all cases the mean clump size is well below ten organisms per air-borne particle.

DISCUSSION

The technique involved in these measurements appears to work well. The principal obstacle to obtaining clear cut results has been the small numbers of pathogenic organisms that can usually be found in an air sample. Since no case of active spread of disease has appeared during the course of the investigation there is no answer to the question posed at the start of this paper: Are those staphylococci which cause disease, and in particular epidemics of staphylococcal disease, present on air-borne particles in larger aggregations than other less 'virulent' strains? It is, however, clear that in the ordinary way *Staph. aureus* is found on air-borne particles in aggregates which usually consist of only a few organisms and rarely contain as many as ten viable cocci.

SUMMARY

The form of the killing curve obtained by bombarding micro-organisms with high energy electrons enables an estimate to be made of the numbers of individual viable organisms present in the aggregates or clumps comprising the sample. Samples of *Staph. aureus* collected from the air of two hospital wards have been found to consist of aggregates containing, on the average, only about four viable cocci per air-borne particle. These samples were taken during quiet periods and during periods of active dispersion of the organism but there was no active spread of staphylococcal disease at any time.

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REFERENCES

BARBER, MARY & KUPER, S. W. A. (1951). *J. Path. Bact.* **63**, 65.
BOAG, J. W., DOLPHIN, G. W. & ROTBLAT, J. (1958). *Radiation Res.* **9**, 589.
BOURDILLON, R. B., LIDWELL, O. M. & LOVELOCK, J. E. (1948). *Studies in air hygiene. M.R.C. Spec. Rep. Ser.* no. 262, pp. 59, 63. London: H.M.S.O.
LIDWELL, O. M. (1959). *J. Sci. Instrum.* **36**, 3.
LINDOP, P. J. & ROTBLAT, J. (1958). *Second International Conference on the Peaceful Uses of Atomic Energy.* Paper 292. London: Pergamon Press.
MORIARTY, J. H. (1950). *Massachusetts Institute of Technology, Dept. of Food Technology, Progress Report,* no. 1, May and no. 2, December.
ROTBLAT, J. (1955). *Nature, Lond.*, **175**, 745.
WILLIAMS, R. E. O. (1959). *Lancet*, i, 190.
WILLIAMS, R. E. O. & HIRCH, ANN (1950). *J. Hyg., Camb.*, **48**, 504.

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