THE RESPIRATORY RETENTION OF BACTERIAL AEROSOLS: EXPERIMENTS WITH RADIOACTIVE SPORES

By G. J. HARPER and J. D. MORTON

Microbiological Research Department, Ministry of Supply, Porton, Wilts

(With 2 Figures in the Text)

In another paper (Druett, Henderson, Packman & Peacock, 1953) the influence on infectivity of the particle size of an aerosol of *Bacillus anthracis* spores is demonstrated. The present paper is concerned with the quantitative study of the retention of particles of different size in the respiratory tract in conditions closely similar to those used in infection experiments with *B. anthracis*.

The degree of retention and site of deposition of inhaled particles have been the subject of numerous investigations. Most of the work on man has been concerned with the pneumoconioses and, more recently, with inhalation therapy: for example, Van Wijk & Patterson (1940), Landahl & Black (1947), Landahl & Herrmann (1948), Landahl & Tracewell (1949), Wilson & La Mer (1948). Summaries of this work have been published by Davies (1949, 1952), and also by Sawyer (1949), who has made further investigations. Work on other mammals has been reported, for example by Scott *et al.* (1949).

We considered that there was no satisfactory substitute for micro-organisms in this work, because retention is affected by the size, shape, density and hygroscopicity of the inhaled particle. For the purpose we chose a strain of *B. subtilis* the spores of which closely resembled the anthrax spores then being used in infection experiments.

No suitable method was available for determining the distribution of the retained aerosol. Barnes (1947) had made successful viable counts on disintegrated tissues; these can be made on the softest organs, using heat-resistant spores, but the process is not easy, and is very difficult and unreliable for determinations on the nasal passages. A further difficulty is the presence of naturally occurring spores in the respiratory tract of animals normally housed. We therefore decided to label the spores with a radioactive isotope. Micro-organisms had previously been labelled by growing them on a radioactive medium, and their distribution after injection had been traced (Ely, 1942), but the level of radioactivity had been far too low for our purposes; it was evident to us that numbers of spores as low as 10^3 must be readily assessed if the technique was to be useful. The first step, therefore, was to develop a method of preparing highly radioactive spores. We then studied the assessment of radioactivity in tissues containing the spores. A preliminary account of this work has been published (Buckland, Harper & Morton, 1950); shortly after this, Goldberg & Leif (1950) published an account of similar work. A detailed account of the preparation and properties of the radioactive spore suspensions has already been published (Harper & Morton, 1952).

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METHODS

Radioactive spores

B. subtilis was grown on CCY agar containing 0·6 mC./ml. of ^{32}P as Na_2HPO_4; the spores were harvested after 45 hr. and washed 6 times. The freshly prepared suspension contained, in each ml., about 2×10^9 spores, of which 20 % were viable; it also contained about 0·05 mC. of ^{32}P, of which more than 95 % was carried by the spores.

Generation of aerosols

Aerosols of single organisms, nominally 1 μ diameter, were generated in the apparatus devised by Henderson (1952), in which a Collison atomizer discharges into a closed circuit fed with dry air; the concentration of cells in the suspension was such that most of the wet aerosol particles would contain no organism and very few would contain more than one, so that after 30 in. travel the aerosol would consist almost entirely of single 'dry' spores. At this point animals were exposed. (Determinations in other experiments indicate that the proportion of single spores always exceeded 90 % by number, and the largest particle was less than 3 μ in diameter.)

Aerosols of larger particles were generated in the apparatus devised by Druett & May (1952), in which the suspension is dispersed from a spinning disk driven at very high speed by air. The 'dry' aerosol consists of uniform particles, of diameter selected as required by adjusting the operating conditions and concentration of suspension. In the present investigation, experiments were done at particle sizes from 2·5 to 12 μ diameter.

Exposure of animals

Guinea-pigs and other small animals were exposed directly to the aerosol passing through either apparatus by thrusting the nose through a hole in a rubber diaphragm; two or more animals were exposed at a time. Monkeys were exposed singly, using a hand-held mask through which the aerosol was by-passed. Exposures were normally made for 1 or 2 min. The aerosol concentration to which the animals were exposed was determined by taking samples in capillary impingers operating under critical flow conditions. The conditions in these experiments closely resembled those in the experiments with pathogens (Druett *et al.* 1953).

Most of the guinea-pigs weighed 350–450 g. The monkeys in each experiment had average weights in the range 8–11 lb., except for the first experiment with 6·1 μ particles, in which they averaged 6½ lb. The mice were in the range 25–30 g.

Preparation of samples

The animals were killed immediately after exposure or later, according to the purpose of the experiment. Guinea-pigs were killed by a blow on the back of the head. In most of the experiments with monkeys, an intraperitoneal injection of Nembutal was used; this meant a delay of about half an hour between exposure and death.

Parts of the respiratory tract, and other organs as required, were rapidly dissected out, radioactive contamination being avoided by precautions such as

careful reflexion and removal of the skin of the face, which had been exposed to the aerosol.

In this paper the following meanings are used:

Head. The complete head, skinned and cut off at about the middle of the cervical vertebrae.

Trachea. From the thyroid cartilage to the bifurcation at the bronchi.

Lungs. The whole of the lungs and vessels including the bronchi.

Stomach. Oesophagus and stomach from pharynx to pylorus.

Gut. From pylorus to rectum.

Body. As defined in the particular experiment.

The selected organs were digested in commercial nitric acid on a sand-bath running at about 200° C. Small organs (e.g. guinea-pig lungs) were treated with 16 ml. of acid in boiling tubes; a monkey's head (10 lb. *Rhesus*) required 250 ml. of acid in a litre beaker. Frothing was occasionally troublesome, but could be controlled by a small addition of DC Antifoam A (Albright and Wilson, Oldbury, Birmingham). The product after cooling was a yellow fluid, with supernatant fat in quantity dependent on the tissues digested, and sometimes a flocculent insoluble material; experiments showed that substantially all the ^{32}P was contained in the fluid.

Assessment of samples

The volume and density of the fluid digest were determined. A 10 ml. volume was counted in an M6 liquid Geiger-Muller (GM) counter (Veall, 1948; Twentieth Century Electronics Ltd.) with conventional scaling equipment, and the count was corrected for the density and total volume of the sample.

Impinger samples, taken in phosphate buffer, were treated with an equal volume or more of nitric acid, heated for 15–30 min. to bring all the ^{32}P into solution, and diluted as necessary in nitric acid of about the same density as the tissue digests. They were counted in the same M6 GM tubes.

PRELIMINARY WORK

The object of the technique was to determine the number of spores located in various organs after exposure to an aerosol in selected conditions. It was therefore necessary to satisfy ourselves that the determinations of radioactivity gave a fair measure of the number of spores present. We attempted to do this by comparing radioactive and viable counts on tissues containing the spores.

(*a*) Guinea-pigs were exposed to aerosols of single radioactive spores in the Henderson apparatus. The lungs from each animal were disintegrated in a specially designed small machine, to give a homogeneous slurry free from large particles of tissue. Serial dilutions were made and 0·5 ml. samples of each were spread on nutrient agar in 6 in. Petri plates which were incubated. The remainder of the slurry was digested with nitric acid and assessed for radioactivity. The relation between viable count and radioactive count of the aerosol was also determined. From this the number of viable spores in each tissue sample could be calculated from the radioactive count and compared with the number obtained directly by

viable count. In four experiments, each with six guinea-pigs, the ratios (found/calculated) and the coefficient of variation (percentage standard deviation) were: 1·28 and 17 %; 1·20 and 7 %; 0·99 and 7 %; 1·03 and 39 %. The tendency to find more spores by viable count is due largely to the natural occurrence of spores in guinea-pigs that have been normally housed; this may also explain the large coefficient of variation in the fourth experiment, which included one very high value.

(*b*) Suspension was instilled directly into the lungs of six tracheotomized guinea-pigs, which were then treated as above. The ratio (found/calculated) and coefficient of variation were 1·42 and 14 %; in a repeat experiment with improved technique they were 1·00 and 13 %.

(*c*) In experiments (*a*) above, the radioactive spore suspension was also centrifuged and six guinea-pigs were exposed to the aerosol sprayed from the supernatant fluid alone. The radioactivity of the aerosol was about 5 % of that from the whole suspension; most of this must have been in water-soluble form, for the viable spore content of the aerosol was only 0·2 % of that from the whole suspension. Only slight radioactivity was detected in the organs after exposure, showing that the supernatant activity (normally less than 5 % of the activity of the whole suspension) was not invalidating the experiments. (See below for determinations of natural radioactivity in guinea-pigs.)

(*d*) Measured volumes of radioactive suspension were added to lungs, head, and other parts of dissected guinea-pigs, which were then digested with nitric acid. The measured radioactivity averaged 90 % of the inoculum.

It was concluded from these experiments that a loss of about 10 % of the ^{32}P was to be expected. This is, we find, in agreement with other workers' experience, and cannot be prevented by obvious means such as addition of inactive phosphoric acid. The correlation between viable and radioactive counts seemed reasonably satisfactory in view of the difficulty of getting good viable counts and the presence of naturally occurring spores, and we felt that the method was sufficiently accurate for the proposed experiments. Experience suggests that the method is better than these preliminary experiments indicated.

RESULTS

The work is in two main parts: experiments on *immediate distribution*, in which the animals were killed 'immediately' and dissected usually within 20 min. of exposure (but see above regarding monkeys); and investigation of *subsequent distribution* when the animals remained alive for up to 24 hr.

The results in Tables 1–4 are expressed in the form of percentage distribution of the measured radioactivity between the various parts of the respiratory tract. The total *inhaled* aerosol, and hence the proportion of it which is retained, could not be determined in these experiments; we have therefore related the recovered radioactivity to volume of aerosol inhaled in a 1 min. exposure, using the measured radioactivity of the aerosol samples and expressing the results as percentages of the normal respiratory minute volume. Guyton's (1947) figures for normal respiratory minute volume have been used. For the guinea-pigs, a respiratory

minute volume of 150 ml. has been taken throughout, corresponding to a body weight of 450 g., though the weights actually ranged from 300 to 500 g.; more precisely calculated figures would not be justified, for it will be seen later that the

Table 1. *Immediate distribution of retained aerosol in guinea-pigs*

Particle diameter (μ)	No. of guinea-pigs	% distribution			ml. aerosol retained per min. as % of resp. min. vol.	
		Head	Trachea	Lungs	Total	Lungs
1	6	28·6	12·9	58·5	75	44
	4	40·5	7·7	51·8	43	22
	4	34·5	11·4	54·1	74	42
	4	39·4	0·9	59·7	75	45
	6	41·5	11·2	47·3	103	49
	6	37·3	22·1	40·6	127	52
2·5	10	54·8 (38·9 + 15·9)*	17·7	27·5	167	46
3·8	6	67·7	19·5	12·8	161	21
4	8	65·2	34·8 (combined)		137	—
4	8	57·5	13·8	28·7	132	38
4·1	6	74·8	16·0	9·2	127	12
5·7	10	94·2 (87·3 + 6·9)	4·2	1·6	113	1·7
6·1	6	87·8	6·6	5·6	109	6
8·4	10	96·5 (88·0 + 8·5)	3·2	0·3	89	0·3
10	8	98·1	0·9	1·0	90	0·8
	8	98·2	1·5	0·3	120	0·4

* The head was divided by a vertical cut just behind the eyes, passing through the straight channel joining the more convoluted front and rear portions, the figures for these being given in brackets thus (Front + Rear).

Table 2. *Immediate distribution of retained aerosol in guinea-pigs. Influence of surface-active agent*

Particle diameter (μ)	No. of guinea-pigs	% distribution			ml. aerosol retained per min. as % of resp. min. vol.	
		Head	Trachea	Lungs	Total	Lungs
1	6	52·4	9·8	37·8	57	21
(5 % 'Tergitol')	6	50·6	7·2	42·2	53	22
4·7	9	75·7 (58·9 + 16·8)	15·5	8·8	117	11
(0·06 % 'Tergitol')	9	79·3 (65·2 + 14·1)	12·6	8·1	97	8

'Tergitol' is a branched long-chain sodium sulphonate of strongly surface-active properties (General Metallurgical Corporation, Holborn, London).

retained volume often exceeds the respiratory minute volume by a substantial margin. The volume for monkeys has been taken as 1440 ml. for 10 lb. body weight; a correction for the mean weights of each group was considered necessary, since these varied widely. The volume for the mouse has been taken as 26 ml. The *total* retention and the *lung* retention have been worked out for each experiment.

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The arithmetical means for each experiment are set out separately in Tables 1–4, since there were differences in the aerosol concentration and time of exposure (to compensate for different specific radioactivity of the spore suspensions used). In Table 5 the results are summarized: the percentage distribution of the total respiratory retention between head, trachea and lungs is given first; figures are given in the last columns for total respiratory retention, and for ingested material, both in terms of millilitres of aerosol and 1 min. exposure. Some standard deviations are included.

Table 3. *Immediate distribution of retained aerosol in monkeys*

Particle diameter (μ)	No. of monkeys	% distribution			ml. aerosol retained per min. as % of resp. min. vol.	
		Head	Trachea	Lungs	Total	Lungs
1	4	34	1	65	27	18
	6	17·5	0·8	81·7	21	17
3·8	6	33·1	4·1	62·8	126	79
4	4	61·6	2·4	36·0	70	25
4·2	6	36·2	17·0	46·8	115	54
5·8	6	51·0	13·1	35·9	173	62
6·1	3	20·9	7·9	71·2	114	81
6·1	3	48·2	4·2	47·6	133	34
11·8	10	84·7	3·5	11·8	75	9

The time of death was about half an hour after exposure.

In the experiment with 11·8μ particles, the stomach contained activity equal to 36% of the total respiratory retention.

Table 4. *Immediate distribution of retained aerosol in mice*

Particle diameter (μ)	No. of mice	% distribution			ml. aerosol retained per min. as % of resp. min. vol.	
		Head	Trachea	Lungs	Total	Lungs
1	8	60·2	3·2	36·6	34	12
	10	59·9	2·0	38·1	19	7

Comments on immediate distribution

(1) The guinea-pig shows the expected change of distribution with particle size: the proportion of material retained in the head increases with increased particle size. A comparison is made in Fig. 1 between our guinea-pig results and those obtained for man by Sawyer, using aerosols of stearic acid spheres (that is, material of similar density to the bacterial spores). The marked agreement encourages confidence in the use of guinea-pigs for experiments on respiratory infection.

(2) The total retention in about half of the experiments exceeds that calculated from the respiratory minute volume; this is probably owing to increased breathing rate consequent upon excitement of the animals. In the guinea-pig, the total retention increases on going from particle size 1 to 2·5μ and decreases on further increase; this may be explained by the more complete retention of larger particles (Sawyer finds for man: 53% at 1μ, 74% at 2μ, and 100% at 10μ), offset at the

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largest sizes by increased deposition at the extreme periphery of the external nares, which in our technique may be partly lost when the head is skinned.

(3) The monkey shows a different picture from the guinea-pig. Although the general trend of increasing head retention and total retention with particle

Table 5. *Distribution of retained aerosol in animals dissected soon after exposure. Results summarized*

		% distribution of resp. retention							Retention as ml. aerosol/min.					
	Particle	Head		Trachea		Lung			Resp. total			Stomach		
Animal species	diameter (μ)	m	n	m	n	m	s	n	m	s	n	m	s	n
Guinea-pig	1	35·7	30	9·5	24	55·2	12·6	24	115	48	24	21·4	48	24
Guinea-pig	2·5	54·3	10	17·1	10	28·9	13·4	10	250	93	10	—	—	—
Guinea-pig	4	60·6	16	14·2	14	30·4	8·1	8	202	55·4	16	11·7	—	12
Guinea-pig	6	90·8	16	5·6	16	3·7	3·37	16	170	81·5	10	1·3	—	6
Guinea-pig	8	95·6	10	4·0	10	0·35	0·2	10	135	48·7	10	—	—	—
Guinea-pig	10	98·1	16	1·4	16	0·64	0·34	16	158	48·8	16	—	—	—
Guinea-pig (with 'Tergitol')	1	52	12	8·5	12	40	—	12	82	—	12	23·3	—	12
	4·7	76·3	18	14·8	18	9·1	—	18	162	43·5	18	—	—	—
Monkey	1	32·4	10	0·76	10	66·8	20	10	301	210	10	27	14·4	4
Monkey	4	43·6	16	8·6	16	47·8	23·5	16	1620	770	16	—	—	—
Monkey	6	42·3	12	8·8	12	48·8	21·8	12	1619	836	12	—	—	—
Monkey	12	86·4	10	3·4	10	10	14·6	10	1081	521	10	391	318	10
Mouse	1	58·8	17	3·0	17	39·5	18·9	17	6·9	3·1	17	—	—	—

m = mean; s = standard deviation; n = sample size.

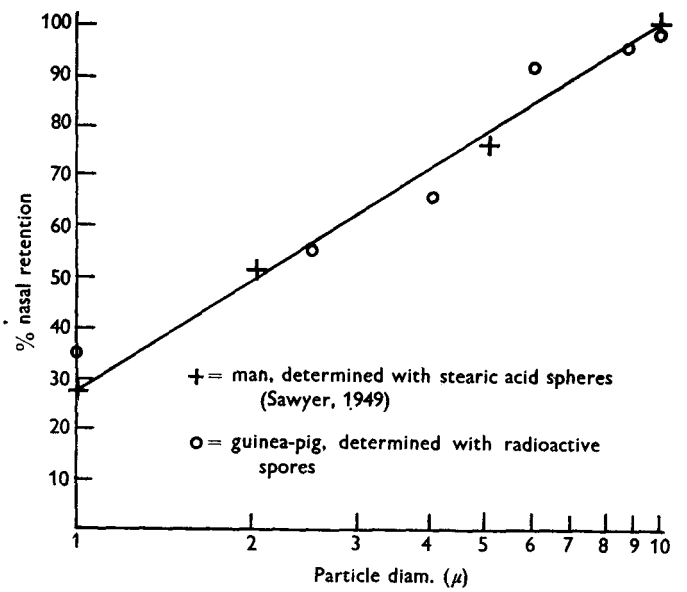

Fig. 1. Percentage of total respiratory retained material found in nasal (head) passages.

diameter is suggested, the results are more scattered; more significant still, the lung retention (expressed as a percentage of the respiratory minute volume) *increases* on going from 1 to 4 μ, and is much the same as 6 μ. We interpret this as being the consequence of an irregular incidence of mouth breathing in the animals,

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which are liable to oppose vigorously the placing of a mask over the nose; the result of mouth breathing will be that particles of 4 or 6 μ diameter, which would have been extensively trapped in the head, are able to pass through the trachea (where there will not be much impaction) and into the lungs, where retention of such particles will be more or less complete. Evidence to support this view is obtained from the relatively large stomach retention, and also by monitoring the skinned head with a β-probe, which often shows heavy radioactive contamination of the mouth.

Subsequent distribution: the influence of time lapse

The ciliated epithelium, which extends throughout the respiratory tract down to the smallest bronchioles, is extremely effective in removing deposited particles, and the rate of removal is high, of the order of 2 cm./min. in mammals. If we are considering insoluble or slowly soluble materials such as pathogens or particles liable to cause pneumoconiosis, it is with the less rapidly removed particles that we are most concerned—probably mainly those in the alveolar ducts and sacs. Experiments were therefore done in which guinea-pigs were exposed and then killed either immediately or after various time intervals.

The technique of exposure was the same as in the other experiments. The animals were exposed in pairs to 1 μ aerosols, and in groups of four to the larger particles. The first batch (usually six) were killed immediately, and the remaining batches were held for the times shown in Tables 6 and 7. Since the dissection took about 5 min. per guinea-pig, the mean time from exposure to dissection was about 15 min. longer than the time from exposure to death.

In the earlier experiments, guinea-pigs not killed immediately were released into cages holding six each. This was a mistake, as was shown by impossibly high radioactive counts from the bodies. We found that radioactive material was being transferred from the heavily contaminated snouts of the animals on to their own and their companions' fur. In subsequent experiments they were held singly in boxes with a neck yoke, and special care was taken to avoid spread of radioactive contamination during dissection.

In Tables 6 and 7, the distribution at zero time has been expressed as in Tables 1–4; that is, the radioactive count for each part has been given as a percentage of the total for the three parts. This cannot be done with the delayed groups, since we wish to see the change in amount retained in each part. The counts have therefore been related to the total respiratory retention at zero time, assuming that the initial retention was the same in all groups of one experiment. This assumption we know to be inaccurate; fortunately, it is possible to apply a correction in most of the experiments, as will be seen later.

Table 8 presents the results in a different way, to show the fate of the spores lost from the respiratory tract. The total GM count for each group of six guinea-pigs is shown, with its distribution among the various parts. The body has not been included. As stated above, spurious results consequent upon contamination were obtained in the earlier experiments. When the technique was improved, body counts were about 5 % of the total and appeared to be independent of time up to

2 hr. The presence of radioactivity in the body is attributed to two causes: the small amount (usually less than 5 % of the total) of ^{32}P in solution in the suspension fluid, which would rapidly pass through mucous membranes into the blood stream;

Table 6. *Distribution of retained aerosol in respiratory tract of guinea-pig 5 and 24 hr. after exposure. 1 μ particles. Four guinea-pigs in each group*

Time after exposure (hr.)	% of total respiratory retention at zero time		
	Head	Trachea	Lungs
0	40·5	7·7	51·8
5	7·0	0·6	60·2
24	14·0	0·4	56·4
0	40·1	7·8	52·1
5	3·8	0·04	27·3
24	7·1	0·04	42·0

Table 7. *Distribution of retained aerosol in respiratory tract of guinea-pig up to about 2 hr. after exposure. Mean time of dissection about 15 min. later than time of killing. Six guinea-pigs in each group*

Particle diameter (μ)	Time of killing after exposure (min.)	% of total respiratory retention at zero time		
		Head	Trachea	Lungs
1	0	41·5	11·2	47·3
	28	6·1	4·3	39·6
	50	7·8	1·1	31·3
	104	3·6	1·3	53·3
1	0	37·2	22·1	40·5
	28	8·6	1·1	44·4
	50	2·7	0·4	34·6
	104	3·8	0·3	35·4
1	0	52·5	9·8	37·8
(5 % 'Tergitol')	28	23·3	1·2	39·7
	50	27·2	1·3	34·2
	104	18·4	1·6	39·0
1	0	50·6	7·2	42·2
(5 % 'Tergitol')	28	32·1	3·4	49·0
	50	32·6	2·4	36·2
	104	21·8	0·4	61·1
3·8	0	67·7	19·5	12·8
	28	50·8	2·0	13·7
	50	17·7	0·7	9·8
	104	21·4	0·3	9·5
4·1	0	75·0	16·1	9·1
	28	31·2	8·5	3·4
	50	17·0	0·6	0·7
	104	17·1	1·3	0·9
6·1	0	87·7	6·6	5·7
	28	68·0	2·0	2·7
	50	61·9	0·9	2·2
	104	38·1	0·3	2·6

and natural radioactivity in the guinea-pigs. Six unexposed guinea-pigs were treated in the usual way and gave the results in Table 9, which includes typical figures from a group of six exposed and killed immediately. It will be seen that

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the radioactive counts in the gut and body at zero time may well be explained by natural radioactivity and soluble ^{32}P in the circulatory system.

The total GM counts in Table 8 may be assumed to be proportional to the total respiratory retention, and therefore afford a means of correcting the figures in

Table 8. *Distribution of retained aerosol in guinea-pig after time lapse. Six guinea-pigs in each group. (The same experiments as in Table* 6)

Particle diameter (μ)	Time of killing after exposure (min.)	Total GM count	% of total GM count				
			Head	Trachea	Lungs	Stomach	Gut
1	0	27,325	34·8	9·4	39·7	16·1	—
	28	24,972	5·6	3·9	36·3	54·2	—
	50	24,403	7·4	1·0	29·4	62·2	—
	104	27,448	3·0	1·1	44·6	51·3	—
1	0	9,191	27·5	16·3	30·0	23·8	2·4
	28	7,439	7·8	1·0	40·5	48·0	2·6
	50	4,587	4·0	0·5	51·1	39·3	5·1
	104	6,521	3·9	0·3	36·8	42·4	16·6
1	0	16,919	38·1	7·2	27·5	25·0	2·2
(5 % 'Tergitol')	28	15,555	18·5	0·9	31·4	47·7	1·5
	50	15,006	22·3	1·1	28·1	46·5	3·0
	104	17,982	12·6	1·1	26·8	44·6	14·9
1	0	18,660	41·4	5·9	34·6	17·7	0·4
(5 % 'Tergitol')	28	25,475	19·3	2·0	29·4	46·7	2·6
	50	21,118	23·5	1·7	26·2	46·3	2·3
	104	26,095	12·8	0·2	35·8	40·9	10·3
3·8	0	11,246	64·3	18·6	12·2	2·4	2·5
	28	9,912	55·7	2·2	14·8	24·5	2·7
	50	8,052	23·5	0·9	13·0	53·3	9·3
	104	6,738	34·1	0·5	15·0	42·1	8·3
4·1	0	17,581	67·1	14·4	8·1	8·1	2·3
	28	15,453	31·8	8·8	3·5	51·2	4·7
	50	12,588	21·2	0·8	0·9	64·2	12·9
	104	15,526	17·4	1·3	0·9	48·7	31·7
6·1	0	16,490	86·8	6·5	5·6	0·8	0·3
	28	14,494	76·4	2·3	3·1	18·0	0·2
	50	12,718	79·4	1·2	2·9	16·0	0·5
	104	12,588	49·3	0·3	3·4	42·5	4·5

Table 9. *Total GM counts in a group of six guinea-pigs not exposed to radioactive aerosol, compared with a typical group of six exposed guinea-pigs*

	Unexposed	Exposed
Head	150	7245
Trachea	Negligible	2083
Lungs	Negligible	1370
Stomach	25	270
Gut	85	278
Body	600	2272

Tables 6 and 7 for variation between groups. The measured total radioactivity has been converted to millilitres of aerosol, and then all groups have been corrected arbitrarily to 150 ml. per guinea-pig per min. These corrected figures have been used for Fig. 2, which shows the percentage change of head and lung retention with time.

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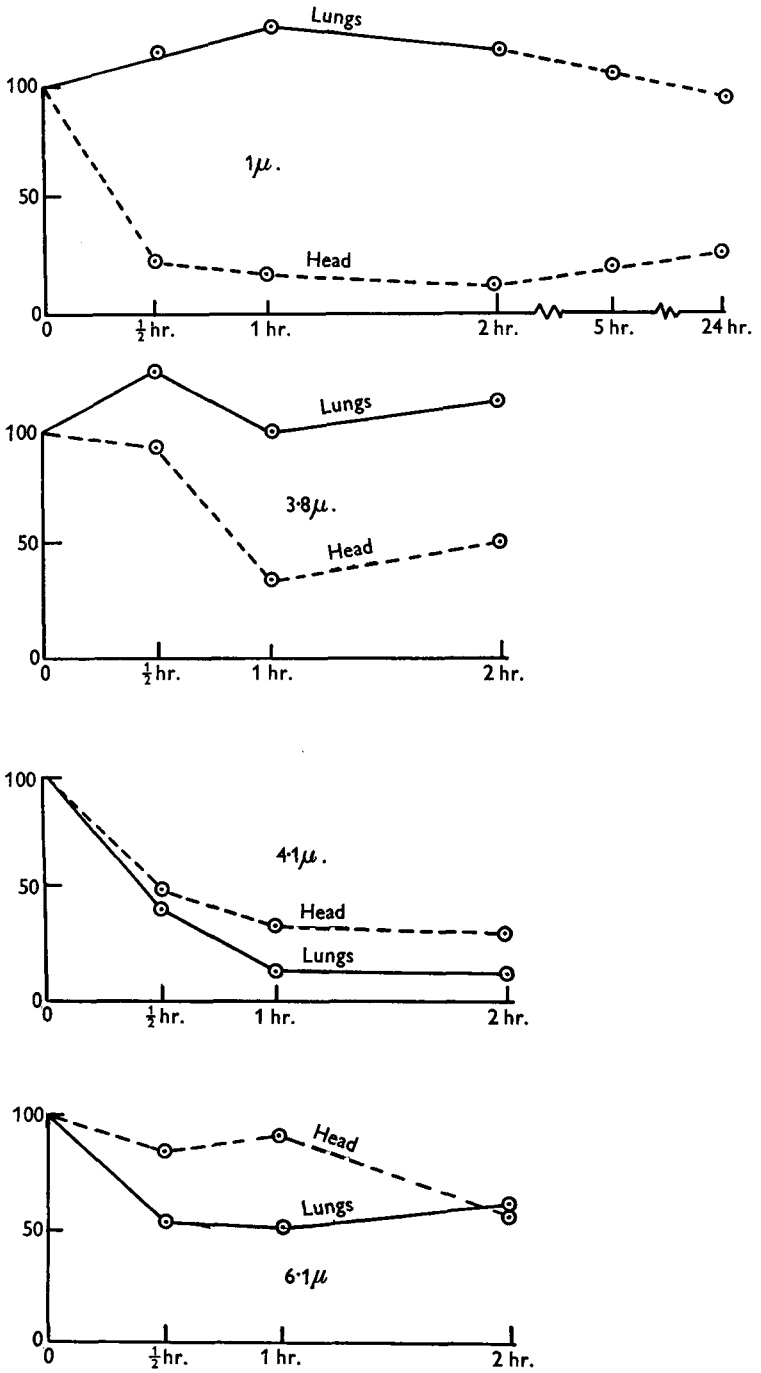

Fig. 2. Percentage change in head and lung retention with time.

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Comments on change of distribution with time

We would expect to find that particles retained on the ciliated epithelium are rapidly removed, being swallowed and passed through the alimentary tract, and that particles retained in the alveoli are not rapidly removed. The results with 1 μ particles show that those which fail to impact in the upper respiratory tract mostly penetrate to the deep recesses of the lungs, for there is little change in lung counts with time up to 24 hr., while particles retained in the head are rapidly (though not completely) removed. When larger particles are used, both the head and lung retentions tend to decrease fairly rapidly, presumably because the 'lung' retention includes a major proportion in the bronchi and bronchioles. It is interesting to note that the head retention decreases less rapidly with the larger particles; evidently the factor responsible for this could also account for the unexpectedly slow removal from the lungs of particles too large to have penetrated as far as the alveoli.

The fate of the removed particles is clearly shown in Table 8, which shows that they pass rapidly into the stomach and later into the gut. The high initial count with 1 μ particles must result from movement of material during the inevitable delay between exposure and dissection, assisted by naturally occurring radioactivity. Note that the stomach activity in guinea-pigs at zero time is much lower with the larger particles, which are deposited mostly in the forward part of the head; and compare this with the monkey, which we believe does a lot of mouth breathing.

The influence of 'Tergitol' was studied because of its power to enhance the respiratory virulence of anthrax spores. It will be seen that it increases the initial head retention, probably because it makes the particles hygroscopic; Sawyer (1949), Druett (unpublished) and others have shown that hygroscopic particles can take up a substantial amount of water while passing through the nasal passages. 'Tergitol' also appears to delay removal, probably by interfering with the physical state of the mucous layer or with the cilia. These observations do not account for the enhancement of virulence, but are important in the interpretation of work now proceeding on that subject.

An alternative presentation of the immediate distribution

Most of the results are given in terms of radioactive counts and their percentage distribution; it is of interest to translate these into actual numbers of organisms and particles. The number of spores for a given radioactive count is calculated from the measured radioactivity and total spore count of the suspension, which were determined shortly before each experiment. The number of spores in a particle of given diameter has been calculated by assuming a spherical spore of 1 μ diameter and a packing fraction of 0·75:

$$\text{No. of spores in particle of diameter } D\mu = 0{\cdot}75\left(\frac{\pi}{6}D^3\right)\Big/\left(\frac{\pi}{6}1^3\right) = 0{\cdot}75\ D^3.$$

Figures for each experiment, calculated in this way, are given in Table 10. The procedure finds some justification when we calculate the number of particles in guinea-pig lungs after exposure to 8·4 and 10 μ aerosols (Table 11). If the experi-

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ments and calculations are sound, these should be integral numbers. It will be seen that fifteen of the twenty-six results are within $\pm 0{\cdot}1$ of an integer; the difference between this and the most probable random value of 2/10 (or 5·2/26) is highly significant. (Note that the packing fraction of 0·75 is appropriate only to large particles and close packing; this has probably been offset by overestimating

Table 10. *Concentration of spores and particles in aerosols, and numbers of these retained in lungs of animals exposed to them. Animals dissected without delay*

Animal species	Particle diameter (μ)	Aerosol concn. $\times 10^{-4}$		Time of exposure (min.)	Lung retention corrected to constant dosage	
		Spores/l.	Particles/l.		Spores $\times 10^{-4}$	Particles
Guinea-pig	1	428	428	2	6·52	65,200
Guinea-pig		220	220	2	3·32	33,200
Guinea-pig		334	334	2	6·87	68,700
Guinea-pig		156	156	5	6·70	67,000
Guinea-pig		237	237	2	8·97	89,700
Guinea-pig		62·8	62·8	2	7·68	76,800
*Guinea-pig		461	461	2	3·22	32,200
*Guinea-pig		314	314	2	3·16	21,600
Guinea-pig	2·5	122	10·2	1	6·87	5,720
Guinea-pig	3·8	102	2·32	2	3·08	700
Guinea-pig	4·0	124	2·58	1	5·70	1,186
Guinea-pig	4·1	141	2·71	2	1·76	339
*Guinea-pig	4·7	19·8	0·254	2	1·60	205
Guinea-pig	4·8	81·7	0·973	2	1·18	141
Guinea-pig	5·7	429	3·11	1	0·288	20
Guinea-pig	6·1	121	0·703	2	0·925	54
Guinea-pig	8·4	731	1·70	1	0·403	0·9
Guinea-pig	10	1400	1·87	1	0·136	1·8
Guinea-pig		302	0·403	1	0·054	0·7
Monkey	1	212	212	1	28·5	285,000
Monkey		110	110	1	17·4	174,000
Monkey	3·8	55·1	1·25	1	125	28,500
Monkey	4·0	292	6·08	1	36·2	7,560
Monkey	4·2	160	2·81	1	77·5	13,600
Monkey	5·8	117	0·808	1	80·8	5,590
Monkey	6·1	407	2·37	1	75·8	4,410
Monkey		295	1·72	1	43·5	2,540
Monkey	11·8	298	0·248	1	13·0	108
Mouse	1	438	438	2	0·309	3,090
Mouse		386	386	2	0·179	1,790

Note. The lung retentions have been corrected to a constant dosage of 10^6 spores/l. at 1 min. exposure.

* With 'Tergitol'

Table 11. *Calculated numbers of particles in individual guinea-pigs' lungs after exposure to aerosols of the given mean particle diameters*

8·4μ		10μ		10μ	
	3·95*		9·0*		4·1*
	5·06*		16·0*		4·1*
	5·06*		28·0*		2·3
	3·38		24·4		1·4
	5·06*		16·5		0·9*
	4·5		18·0*		0·5
	6·76		34·9*		0·9*
	6·76		38·4		2·3
	11·9*				
	9·02*				

* Values within $\pm 0{\cdot}1$ of an integer.

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the unit cell occupied by a spore.) It has been pointed out to us that this result depends on an unexpectedly high degree of homogeneity in the aerosols; it is, nevertheless, on statistical grounds, unlikely to be a matter of chance.

SUMMARY

The distribution of inhaled bacterial aerosols has been studied in guinea-pigs, monkeys and mice, using *Bacillus subtilis* spores labelled with radiophosphorus. Particle sizes from about 1 to 12 μ have been used.

The guinea-pig shows the expected change of distribution with particle size; the proportion retained in the head increases with increased particle size. The figures correspond closely with those for man. Monkeys show similar results but are more irregular.

The subsequent fate of the retained particles has been studied and accords with what is known about ciliary removal.

The work is intended to link with parallel investigations of respiratory infection in closely similar conditions, and its implications are discussed in a paper on that subject (Druett *et al.* 1953).

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