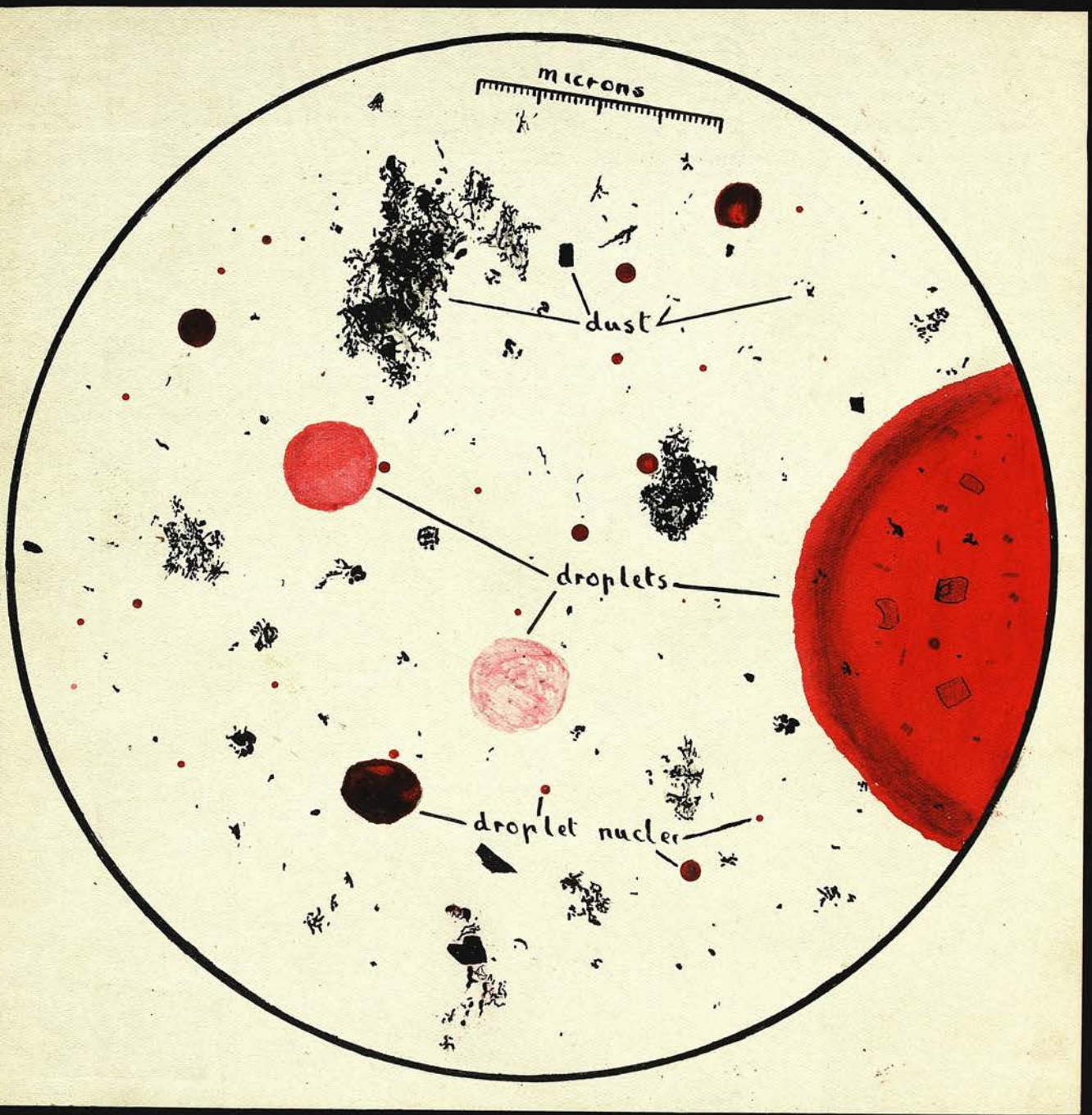
microns
dust
droplets
droplet nuclei

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M.D. 1929

Vol. 2.

A STUDY OF THE MECHANISMS OF TRANSMISSION OF RESPIRATORY-TRACT PATHOGENIC BACTERIA.

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PARTS 2-6:- EXPERIMENTAL.

CONTENTS

Part 2

EXPERIMENTAL INVESTIGATION OF THE PHYSICAL CHARACTERISTICS OF RESPIRATORY DROPLET-SPRAY WHICH RELATE TO THE TRANSMISSION OF INFECTION

CONTENTS OF PART 2

(16)/

(16) Estimation of droplet numbers by method (ii): counting by microscopical observation the deposit marks of dye-containing droplets caught on celluloid slides exposed directly to spray in front of mouth.

(17) Estimation of droplet numbers by method (iii): counting colonies on blood agar plates exposed in slit sampler for collection of droplet nuclei from air of chamber contaminated with spray.

(18) Estimation of droplet numbers by method (iv): counting by microscopical observation the dye-containing droplet-nuclei on oiled slides exposed in slit sampler for collection of nuclei from air of chamber contaminated with spray.

(19) Counting droplet-nuclei by the cultural method (iii) and the microscopical method (iv) employed in parallel.

(20) Differential counting of droplets originating from the throat and droplets originating from the nose.

(21) Micrometric measurement of deposit marks of dye-containing droplets caught on celluloid slides exposed directly to spray in front of mouth.

(22) Calculation of diameters of spherical parent droplets from measured diameters of droplet deposit marks.

(23) Micrometric measurement of dye-containing droplet nuclei collected from air of a chamber contaminated with droplet spray on to oiled slides exposed in slit sampler.

(24) Micrometric measurement of dye-containing droplet nuclei collected on to exposed slides by sedimentation from air of chamber contaminated with droplet spray.

(25) Estimation of diameters of unevaporated parent droplets from measured diameters of dye-containing droplet-nuclei.

(26) Construction of a composite size-distribution series for the droplets of all sizes expelled in speaking, in coughing and in sneezing.

(27) Calculation of the proportion of droplets in each size group which will contain commensal or pathogenic micro-organisms.

(28)/

(28) Counting by microscopical observation the commensal bacteria in droplets of each size.

(29) Calculation of the proportion of droplets and nuclei of all sizes which will contain commensal or pathogenic organisms.

(30) Estimation of the efficiency of the different droplet counting methods by consideration of the observed and composite size-distributions.

(31) Observation of the duration of air carriage and the sedimentation rate of droplet-nuclei by repeated slit sampler examinations of the air of a chamber contaminated with droplet spray.

(32) Calculation of sedimentation rate of droplets and nuclei from counts made microscopically on slides exposed on the floor at 5 feet below the mouth at various intervals after spray production.

(33) Observation of time required for droplet evaporation and droplet-nucleus formation.

Section 34: TABLES, GRAPHS AND FIGURES.

Sections 35-41: DISCUSSION OF FINDINGS.

(35) Comparison of the sanitary significance of the different expiratory activities in terms of the number of droplets expelled by each.

(36) Site of origin of the respiratory tract secretion droplets.

(37) Size of secretion droplets and nuclei.

(38) Proportion of secretion droplets and nuclei containing commensal and pathogenic organisms.

(39) Sedimentation rate and duration of air carriage of secretion droplets and droplet-nuclei.

(40) Time of evaporation of secretion droplets. Droplet-nucleus formation.

(41) Droplet projection distance. Range of infection.

Section 42: CONCLUSIONS.

Section 43: SUMMARY.

Section 44: REFERENCES.

Section 1: Purpose of the investigation.

The investigation was concerned with those physical and mechanical characteristics of respiratory droplet spray which determine the facility of the spray for transmitting infection. Information was sought concerning: (1) the microscopic appearance of droplets and droplet-nuclei, (2) the numbers of droplets and nuclei produced by the different kinds of expiratory activity, (3) the proportions of droplets and nuclei which originate from the different localities of the respiratory tract, (4) the sizes and size-frequency distributions of the droplets and nuclei, (5) the proportions of droplets and nuclei containing commensal bacteria and the number of commensal bacteria in droplets and nuclei of each different size, (6) the proportions of droplets and nuclei likely to contain pathogenic bacteria, (7) the duration of air carriage and the sedimentation rate of droplets and nuclei of each different size, (8) the projection distance of droplets and the travelling distance of nuclei, (9) the time required for droplet evaporation and droplet-nucleus formation, and (10) the maximum size of droplet capable of becoming a droplet-nucleus.

Most of these physical characteristics exert an important influence on the concentration of droplets and nuclei containing pathogenic organisms which will reach the environment of exposed persons. This environmental/

environmental concentration of infected particles is probably one of the main factors in determining the incidence of clinical disease. It would seem that the incidence of infective disease due to droplet spray will vary in direct linear proportion to the environmental concentration of infected droplets and nuclei, and this in turn will vary in direct linear proportion to the numbers of droplets and nuclei produced, to the proportion of these containing pathogenic organisms, to the duration of air carriage of the droplets and nuclei, and to the projection distance of the droplets and the travelling distance of the nuclei. Obviously, the number of infected droplets and nuclei present at a given distance from the source of the spray at a given time after its emission, will depend on the number of infected droplets and nuclei produced, the distance which they can travel and the time for which they can remain airborne.

The size of droplets and nuclei exerts an important influence on the environmental concentration of infected particles; it does so in two different ways. In the first place, the proportion of droplets containing pathogenic organisms and the number of pathogenic organisms per droplet varies in direct proportion to the droplet volume, that is to the cube of the droplet diameter. In the second place, the duration/

duration of air carriage decreases with increasing size. The duration of air carriage of a droplet nucleus varies inversely with its sedimentation rate and thus, according to Stokes' law, inversely with the square of its diameter (for example, a 4-fold decrease in diameter would give a 16-fold increase in duration of air carriage). Droplet size exerts its greatest influence on duration of air carriage in the range where it determines whether or not a droplet can become a droplet nucleus; as its diameter is about 1/5th that of the parent droplet, the nucleus can remain airborne for about 25 times longer (for example, calculation from figures given by Wells, 1934, shows that a water droplet 0.2 millimeters in diameter would, before evaporating, fall the height of a man, 2 meters, in 1½ seconds, while a droplet of 0.05 millimeters in diameter would within ½ second fall 0.3 meters and evaporate to a 0.01 millimeter nucleus which would fall the remainder of 2 meters in 500 seconds; thus, in this size-range a 4-fold difference in diameter entails a 300-fold difference in duration of air carriage). The size of droplets and nuclei has a further importance since it determines whether they are likely, when inhaled, to be caught in the upper respiratory passages or to penetrate to the lung alveoli.

The site of origin of the droplets is important. The various localities of the respiratory tract differ markedly in their microbial flora and thus the site of atomisation/

atomisation influences the proportion of the droplets liable to contain pathogenic organisms.

There is still need for adequate information about droplet numbers, sizes and air carriage times. The observations so far recorded by other investigators have not given a complete or proper account of droplet spray. The methods previously used for collecting and observing droplets were capable of revealing only a small and unrepresentative proportion of the whole. In comparison with the numbers revealed by a new method in the present investigation, the previous methods revealed only about 10% (see Table 7).

The concept of droplet nucleus formation was elaborated by Wells (1934) on a purely theoretical basis. Subsequently, several investigators were able to demonstrate bacterial contamination of air which presumably was due to the presence of bacteria-carrying droplet nuclei. Apart from this indirect evidence for its existence, the droplet nucleus has remained a hypothetical entity. Its direct demonstration by a microscopical method is therefore of considerable interest, and this has been achieved in the present investigation. Furthermore, for confirmation of the values derived theoretically by Wells, an attempt has been made to discover experimentally the maximum size of droplet capable of becoming a droplet nucleus, and the time required for droplet nucleus formation.

Section 2: Methods employed for observation. Difficulties of observation.

It must be emphasised that there is not any entirely satisfactory method for observing droplet spray. This is a consequence of the heterogenous composition and transient existence of the spray. The droplets emitted during a single expiratory act vary greatly in size, projection speed, projection direction, evaporation rate and bacterial content; the droplets 'disappear' by evaporation, dispersion and sedimentation within a second after leaving the mouth. The amount of spray varies very greatly between separate expiratory acts of the same kind, as between one sneeze and another, making it impossible to obtain constant amounts of spray in successive experiments.

The greatest difficulties in studying droplet spray arise from the extreme variation in size among the droplets, for instance from 1 to 2000 microns in diameter (see Table 15). This diversity of sizes renders it impossible by any single method to collect and observe all, or even a representative majority, of the droplets expelled during a single expiratory act. The methods which are efficient for collection of the large and medium sized droplets, fail to collect the small droplets (under 20 microns in diameter), while the methods which are efficient for collection of the medium sized and small droplets, fail to collect the large droplets (over 100 microns in diameter). Thus,

to/

to obtain a comprehensive account of droplet spray, to ascertain the total number and size-distribution of the droplets, it is necessary to employ at least two methods of collection, one efficient for the large droplets and another for the small, and to combine the two sets of results. This procedure has been attempted for the first time in the present study. The calculations required for proper collation of the results allow the possibility of considerable error. However, there is no other way of obtaining a comprehensive account of droplet spray, and for this reason the procedure of collation was undertaken.

A variety of methods are available for collection and observation of droplets and nuclei. All of these methods except one, the photographic method of Jennison and Edgerton (1940), have been employed in the present investigation. The methods fall into three groups: (1) Observation of droplet spray by dark-ground illumination. The droplets are not collected. They are observed while travelling forwards through the air. Either a) the droplets are observed directly with the naked eye while illuminated obliquely by sunlight against a dark background, or b) the droplets are photographed while illuminated obliquely by a flash of light (Jennison and Edgerton, 1940). The photographic method is the better, but even it does not reveal droplets smaller than about 10 microns in diameter.

(2)/

(2) Collection of droplets on culture plates or microscope slides exposed in the path of the droplet spray directly in front of the mouth and nose. The large and medium sized droplets have a high momentum which carries them out of the deflected air stream to impinge upon the opposed plate or slide, but the small droplets (under about 20 microns in diameter) have insufficient momentum, are carried past the plate or slide in the deflected air stream, and so are not collected (see Table 16 and Graphs 3-5). (3) Allowing the droplets to evaporate and become droplet-nuclei while airborne in a closed chamber, and then collecting the nuclei from the air on to a culture plate or microscope slide either by allowing prolonged sedimentation or by use of a sampling device. The small and medium sized droplets are collected by this method, but the large droplets (over 100 microns in diameter) do not remain airborne as nuclei and so are not collected.

After collection by the methods just described, the droplets and nuclei are observed in one of two ways: a) By cultural demonstration; the droplets or nuclei are collected on a plate of culture medium. The colonies developing on this plate are counted and their number is taken to represent the number of droplets or nuclei. The method fails to reveal the droplets and nuclei which do not contain commensal bacteria/

bacteria capable of growing on the culture medium used. This is a serious deficiency; it results in about 90% of the droplets and nuclei being missed (see Table 19). However, the majority of previous investigations have been made by use of this method because it is technically easy and because the extent of its deficiencies was not appreciated. b) Microscopical demonstration. In order that the droplets and nuclei will be clearly visible, the mouth secretions are colored by a dye taken into the mouth prior to the production of spray. The droplets or nuclei are collected on a microscope slide and examined microscopically. Microscopical demonstration is preferable to cultural demonstration since it does not depend on the presence of viable bacteria in the droplets and nuclei; for this reason it gives higher and more accurate counts. Microscopical demonstration has the additional advantage that it makes possible measurement of the droplets and nuclei.

The method whereby dye-containing droplet-nuclei are collected from the air and observed microscopically was developed and used for the first time in the present investigation. It gave higher counts than any other method and yielded much new information about the size-distribution of droplets and droplet-nuclei.

Section 3: Degree of accuracy attained and degree of accuracy required.

The investigation was concerned with the physical characteristics of droplet spray and was carried out by procedures of enumeration, measurement and computation. It might be expected that results would be obtained with the high degree of accuracy usual in the mensuration practice of modern physical science. In fact, only a low degree of accuracy could be achieved. However, a high degree of accuracy was considered unnecessary for the present purpose of evaluating the hygienic significance of droplet spray.

Reference has been made to the difficulties of studying droplet spray; these make necessary the employment of methods of observation which are capable of yielding only approximate results. Because the number of droplets produced by an expiratory act is often very large, it is usually necessary to count and measure only a small portion of the whole and to compute the whole by an appropriate multiplication; this procedure entails the errors inherent in "random sampling". The available methods of collecting droplets and nuclei, whether by sedimentation, impingement or use of a special sampling device, are of indeterminate efficiency for droplets of certain sizes. Furthermore, the best method available for observation of small droplets and nuclei, the microscopical method, can not give information about any droplets or nuclei which may be smaller than

¼ micron in diameter, this being the limit of microscopical resolution. The size of droplets can not be measured directly, but must be calculated from measurements of droplet nuclei or from measurements of droplet deposit marks; the assumed ratios between these measurements and droplet size can be estimated only approximately. The amount of spray varies greatly between separate expiratory acts of the same kind; this renders uncertain the significance of the average counts and size-distributions which were calculated from a dozen or so experiments with each kind of activity.

A detailed analysis of the probable errors of each method of observation was not attempted. A brief consideration suggested that the errors of the initial observations, counting and micrometrical measurement, were probably of the order of 10%. The errors of assumption involved in derivation of values by calculation must have been much greater, but probably were seldom more than 2-fold (i.e. plus 100% or minus 50%).

It is considered that results liable to errors of up to 2-fold are still sufficiently accurate to allow proper and adequate evaluation of the sanitary significance of droplet spray. Any error in the values obtained for numbers, sizes, sedimentation rates or projection distances, is unimportant if it represents/

represents a variation in droplet spray which would entail only an insignificant variation in the incidence of clinical infection. The sporadic and epidemic incidences of specific respiratory infections frequently vary within short periods of time to the extent of 2-fold, 10-fold, or even 100-fold. If it is accepted that variations in infection rate are important only if 2-fold or greater, it may be concluded that variations in the number of droplets, the proportion containing pathogenic organisms, the duration of air carriage and the range of travel are important only if 2-fold or greater, since infection rate is presumably related to these factors in linear proportion. Thus, if the true number of droplets emitted is 100, an error giving counts of 99, 90 or even 60, would be of trivial importance, while an error giving counts of 20, 10 or 1, would be serious. Greater accuracy is required in estimating droplet size since the environmental concentration of infected particles varies, not in linear proportion, but with the square or cube of the droplet diameter; thus the minimum significant variation in droplet diameter would be about 1.3-fold instead of 2-fold.

In the Tables of Results, most of the derived numerical values are given as corrected to two significant figures; this is not intended to represent the degree of accuracy of the methods of derivation.

Section 4: Expiratory activities examined.

The following expiratory activities were examined: (1) nose breathing for one- and five-minute periods; (2) mouth breathing for a one-minute period; (3) loud simulated laughing for a one-minute period; (4) speaking loudly 100 "K's" in words such as "cake" and "kick" which contain no other different consonant; (5) speaking softly 100 words by counting from "one" to "a hundred"; (6) speaking loudly 100 words by counting from "one" to "a hundred"; (7) single 'throat-only coughs', voluntarily produced with the mouth well open and the tongue depressed; (8) single 'lip coughs', voluntarily produced with the mouth at first closed by approximation of the lips and the air blast then forced suddenly out between these; (9) single 'tongue-teeth coughs', voluntarily produced with the mouth at first closed by approximation of the tongue and upper teeth and the air blast then suddenly released between these; (10) single 'natural sneezes', induced by tickling the nasal mucosa with a cotton wool swab; (11) single 'simulated sneezes', voluntarily produced by forming explosively the sound "ttsch"; and (12) single strong nasal expirations of the type made normally to clear minor obstruction or irritation. On some occasions the coughs were tested in volleys of 5 to 50 at a time and the average count calculated.

Section 5: Persons acting as test subjects for droplet spray production.

Most of the experiments were carried out with the same test subject, a male adult who was free from respiratory infection (subject A). Certain kinds of experiments were repeated with each of five other healthy adults (subjects B,C,D,E,F).

Section 6: Air sampling devices.

Air was examined for droplet nuclei mainly by use of a slit sampler constructed according to the design of Bourdillon, Lidwell and Thomas (1941). This device was chosen because it seemed to be the most efficient and most convenient sampler available. Bourdillon and his colleagues claim that the slit sampler recovers more than 94% of the smallest bacteria-carrying particles present in air, and an even greater proportion of the larger particles. The slit sampler was run so as to take 1 cubic foot of air per minute. The distance of the slit above the surface of the culture medium or of the receiving slide was in all cases 2 millimeters. A second slit with chamber for holding a microscope slide was constructed for use when two samples had to be taken in close succession.

In a few experiments when further samplers were required, use was made of two sieve plate samplers constructed, with modification, from the design of Du Buy and Crisp (1944).

Section 7: Chambers used for air contamination experiments.

Four differently-sized chambers were used for the experiments on air contamination. Their capacities were, respectively, 1700 cu.ft. (8 ft. high x 15 ft. x 14 ft.), 100 cu.ft. (8 ft. high x 3½ ft. x 3½ ft.), 70 cu.ft. (5 ft. high x 4 ft. x 3½ ft.) and 2½ cu.ft. (1½ ft. high x 1 1/3 ft. x 1¼ ft.).

The 1700 cu.ft. chamber was a small room with minimal ventilation. Its window and ventilator were sealed; its door was fitted with draught excluders. The test subject, the observer and the slit sampler were all present within the 1700 cu.ft. chamber during experiments. The slit sampler intake was 3 ft. 4 in. above the floor.

The three smaller chambers were used within the 1700 cu.ft. chamber. They were large boxes of wood and plaster board. They were closed and airtight except for a small vent through which air was withdrawn to the slit sampler outside and a "door" through which droplet spray was introduced. The test subject, the observer and the slit sampler remained outside these smaller chambers.

The 100 cu.ft. chamber was fitted in one corner of the 1700 cu.ft. room. It had a full-sized, close-fitting door; sometimes the test subject entered the chamber, but usually he stood outside, opened the door a/

a little, introduced droplet spray and immediately closed the door. Air was withdrawn to the slit sampler through a vent in the chamber wall 3 ft. 4 in. above the floor. Figure 10 shows the 100 cu.ft. chamber, its door, the slit sampler outside and the curved glass tube joining the chamber vent to the intake of the slit sampler.

The 70 cu.ft. chamber had a small "door" which was 6 inches square and situated at the top of one side of the chamber, between 4½ ft. and 5 ft. above the floor; after introduction of droplet spray, the "door" was closed and kept closed. The vent for air sampling was situated in the chamber wall at 3 ft. 4 in. above the floor.

The 2½ cu.ft. chamber also had a small "door" situated at the top of one of its sides, between 1¼ ft. and 1½ ft. above the chamber floor. The vent for air sampling was at the level of the chamber floor.

The inside surfaces, the walls, floor and roof, of the test chambers were coated with spindle oil in order to minimise air contamination by the liberation from these surfaces of bacteria-carrying dust particles.

Wet and dry bulb temperature readings were taken in each experiment, and the relative humidity computed.

The ventilation rates in the test chambers were not measured. In ordinary rooms there are usually between/

between 1 and 20 overturns of air per hour. As the test chambers were closed and sealed to minimise ventilation, their ventilation rate was almost certainly less than 1 overturn per hour and probably nearer 0.1 overturns per hour.

Air circulation times within the chambers were not measured. In the larger chambers air circulation time was probably of the order of a minute as in ordinary rooms.

One or more days was allowed to elapse between successive experiments in the same chamber so that any residual contamination of the air might be eliminated completely by sedimentation and ventilation.

Section 8: Collection of droplets on culture plates or microscope slides exposed directly in front of mouth and nose.

The position and distance from the mouth at which plates and slides should be exposed so as to catch the greatest proportion of droplets, was decided after careful consideration of the photographs taken by Jennison (1942). These photographs show that in speaking, coughing and sneezing the droplets diverge widely and scatter as they pass forward from the mouth, much as do the drops in the spray from a watering can rose. It seemed from the photographs that to avoid missing any portion of the spray a 12 sq.in. culture plate would have to be held very close to the mouth, certainly not more than 3 inches away. On the other hand, the closer a plate were held to the mouth the greater would be the chance of confluent growth resulting instead of separate countable colonies. A distance of 3 inches was chosen for exposure of the culture plates. The plate was held with the surface of the medium facing the mouth and lying parallel to the plane joining the tip of the nose to the tip of the chin. It was considered that the entire droplet spray would be caught on a culture plate held in this way.

It was not practicable to catch the entire spray on a single microscope slide whatever the position and distance from the mouth. The slide was exposed at 6 inches in front of the mouth, with its surface in the vertical plane and facing the mouth.

Section 9: Collection of droplet nuclei from air of chamber on to culture plates or microscope slides by use of a slit sampler.

The droplet nuclei were allowed to become evenly distributed throughout the air of a closed chamber and a known proportion of this air was sampled with the slit sampler. The 1700 cu.ft, the 70 cu.ft. and the 2½ cu.ft. chambers were used. In tests of sneezing it was convenient to use the larger chambers and so obtain a great dilution of the very numerous droplet nuclei. In tests of coughing and speaking it was more convenient to use the smaller chambers and thus maintain a high concentration of the less numerous nuclei. In the larger chambers, of 1700 cu.ft. and of 70 cu.ft., an electric fan was run at half speed to ensure quick and thorough distribution of the nuclei; the droplet spray was directed into the fan air-stream from standing height, 5 feet above the floor. In the case of the 2½ cu.ft. chamber, distribution of the nuclei by an electric fan was considered unnecessary; the droplet spray was introduced horizontally through the "door" at 1½ feet above the chamber floor.

Section 10: Cultural demonstration of droplets and nuclei. Counting of colonies.

The culture medium usually employed was blood agar: 5% horse blood, 1% meat extract, 1% peptone, ½% sodium chloride and 2% agar; this medium was chosen as being suitable for growth of the majority of the bacteria resident as commensals in the secretions of the respiratory tract. The medium was held in Petri dishes of 3⅞ inches diameter, the exposed surface of the medium in each dish having an area of 12 square inches. The culture plates were exposed directly to the droplet spray or were exposed in the slit sampler to the air of a chamber contaminated with spray. The plates were then incubated aerobically at 37°C. for 48 hours. The colonies were larger and easier to distinguish after incubation for 48 hours than after incubation for only 24 hours; slightly larger counts were obtained after the longer period. 'Spreading' bacteria were not encountered in the respiratory tract flora, so that it was unnecessary to curtail incubation in order to prevent spoiling of the plates. To facilitate counting, the surface of the agar in each dish was scored by about half a dozen parallel cuts with a scalpel. The colonies were observed and counted with a binocular plate microscope (magnification of x 1000); the microscope was considered necessary for sure recognition of the smaller colonies, and slightly greater counts were obtained by its use than by counting with the naked eye.

Section 11: Microscopical demonstration of droplets and nuclei. Use of a dye to colour the mouth secretions. Micrometrical measurement.

As an alternative to methods involving cultural demonstration, droplets and nuclei were counted by direct microscopical observation. The mouth secretion was colored with a dye to ensure that even the smallest droplets and nuclei would be readily visible and easily distinguished from dust particles. The dye used in the majority of experiments was Congo red. About 0.1 grams of Congo red powder was applied with a throat swab to the surfaces of the mouth and fauces. Heaviest application was made to the tip of the tongue, the front teeth and the lips, since droplet spray originates mainly from the secretions of the anterior part of the mouth. Congo red was not introduced into the nose. Salivation was usually increased because of the bitter taste of the dye; excess saliva was swallowed before spray production. Colorimetric observations of samples of saliva removed from the mouth showed that the concentration of Congo red varied between 1% and 4%, and was usually about 2%.

In a few experiments eosin powder or liquid India ink was taken into the mouth instead of Congo red. Sometimes about 0.1 grams of eosin powder was used and sometimes about 0.01 grams. The larger quantity of dye, 0.1 grams, whether of eosin or of Congo red, was necessary so that the smallest droplet nuclei might be colored/

colored intensely enough for ready recognition. Use of this larger quantity had the disadvantage that the the solid content of the saliva was greatly increased (e.g. from 1% to 3%) so that the droplet nuclei would be unnaturally large (e.g. 1/3 or 1/4 instead of 1/5 of the parent droplet diameter). When only 0.01 grams of eosin was taken into the mouth, the solid content of the saliva was little increased (e.g. from 1% to 1.2%) so that the droplet nuclei would be nearly their natural size; 0.01 grams of eosin was sufficient to colour all but the smallest nuclei intensely enough for easy recognition.

After about half a minute had been allowed for solution of the dye in the mouth secretions, the sneezing, coughing or speaking was begun. The dye-containing droplets and nuclei were collected from the air on to a microscope slide and were observed under the microscope with the low power (2/3 in.), high power (1/6 in.) and oil-immersion (1/12 in.) objectives. The slide was manipulated with a mechanical stage, this being essential for its regular scanning in parallel strips. When the slide was observed under the microscope, moisture from the observer's breath condensed on all parts of its surface not covered with immersion oil. The droplet nuclei lost dye by solution into this condensation water; the characteristic appearance of the nuclei was destroyed and large colored marks were produced which resembled droplet/

droplet deposit marks. To prevent this falsification of appearance, the droplet nuclei were protected by covering the entire surface of the slide with immersion oil prior to microscopical observation of any kind, even with the "dry" objectives. Direct application of immersion oil on to the droplet nuclei did not give rise to any difficulty; it did not disturb the nuclei or alter their appearance.

Droplet deposit marks and droplet nuclei were measured by use of an eyepiece incorporating a micrometer scale. This scale was seen superimposed on the microscope field (see Figures 5 and 6). It consisted of 10 major divisions and 100 minor divisions. The field distance corresponding to a minor division was determined by comparison with a standard slide micrometer scale. For the microscope used, a minor division of the eyepiece scale corresponded to 17 microns in a field of the low power (2/3 in.) objective, to 3.4 microns in a field of the high power (1/6 in.) objective and to 1.7 microns in a field of the oil-immersion (1/12 in.) objective. When, as was usual, the droplet mark or nucleus possessed a fairly even and circular circumference, there was no difficulty in measuring the diameter. When the circumference was oval or irregular, the maximum and minimum diameters were measured and the mean diameter calculated; the results were recorded in terms of equivalent circular diameters in the case of droplet marks and equivalent spherical/

spherical diameters in the case of droplet nuclei. When large numbers of droplets or nuclei were measured for compilation of a size-distribution series, the actual measurement for each individual droplet or nucleus was not recorded. A table of size-groups was made and as each individual was measured its presence was recorded in the appropriate size-group. Thus, instead of a droplet nucleus being recorded as 5.5 microns in diameter, it was recorded as being in the 4-6 microns diameter group. This procedure made possible the very rapid measurement of large numbers of droplets and nuclei.

Section 12: Naked-eye observation of droplet spray in sunlight.

The test subject was stationed near a window through which bright sunlight was shining; he expelled droplet spray towards the window. The observer was stationed so that he could watch the spray against a dark background within the room. The expelled droplets were easily and clearly visible to the observer who could see whether they were numerous or few, how far and in what direction they travelled, and whether they fell to the ground or remained airborne.

Almost certainly the smallest droplets and nuclei were too small to be seen by the naked eye, so that a considerable proportion must have been missed. It is probable, however, that the minimum size visible to the naked eye by sunlight dark-ground illumination was not so very much greater than the minimum size revealed by the photographic method of Jennison (1942), of about 5 to 10 microns diameter. In the present study by observation with the naked eye, airborne droplet nuclei were seen which did not show any tendency to settle downwards and so could not have been larger than about 10 to 20 microns in diameter. According to the calculations of Wells (1934) and according to measurements made in the present investigation (see Tables 11 and 47), 25 microns diameter is about the upper size limit for droplet nuclei. The nuclei seen in sunlight with the naked eye must have been smaller than/

than this; their imperceptibly slow sedimentation rate suggests that they were nearer 10 microns in diameter.

Sneezing was seen to be much the most prolific droplet producing activity; enormous numbers of droplets were expelled both by simulated sneezes and by 'natural sneezes', the larger numbers by the former. Moderate numbers of droplets were expelled by coughs made with the mouth initially closed, that is by 'lip coughs' and 'tongue-teeth coughs'. In contrast, coughs made with the mouth kept well open, 'throat-only coughs', were not seen to expel any droplets. Small numbers of droplets were expelled in speaking. Very few droplets, or none at all, were seen to be expelled in speaking in a normal conversational tone with the lips dry. A considerable number of droplets were expelled only when certain consonants were spoken forcefully and usually only when the lips were wet or when there was abundant saliva about the front teeth. Thus, according to forcefulness and "wetness", there was great variation in the numbers of droplets emitted in speaking. The majority of the expelled droplets were produced by the consonants "B", "P", "F", "V", "D", "T" and "S". The consonants "B", "P", "F" and "V" usually gave a considerable number of droplets when pronounced loudly with the lips wet and a few droplets when pronounced quietly with the lips wet. When pronounced loudly with the lips dry, "B" and "P" did not/

not give any droplets, while "F" and "V" sometimes gave a few. The consonants "T", "D" and "S", whether the lips were wet or dry, usually gave a considerable number of droplets when pronounced loudly and only sometimes gave a few droplets when pronounced quietly. The consonants "J", "K", "L" and "R" occasionally gave a few droplets when pronounced loudly with the lips either wet or dry. The consonants "G", "H", "M", "N", "Q", "W", "X", "Y" and "Z", and the vowels, all failed to give any visible droplets, whatever the manner of pronunciation.

The projection distance of the droplets was observed. This was greatest for sneezes, less for coughs and least for speaking. For a single expiratory act of any kind, the expelled droplets showed great variation in projection distance; the majority were projected for distances ranging from a few inches to about 3 feet. A few large droplets were seen to travel further than the majority, passing rapidly forwards and downwards to reach the floor at about 1 second after leaving the mouth; this time of 1 second for falling through 5 feet to the floor was measured with a stopwatch on several occasions. The maximum observed projection distance of a large droplet was 10 feet for simulated and 'natural' sneezes, 8 feet for 'tongue-teeth coughs', 4 feet for 'lip coughs' and 2-3 feet for speaking.

Droplet/

Droplet nucleus formation was best seen in the abundant spray produced by a simulated sneeze. Within a fraction of a second after the sneeze, a dense cloud of droplets was seen extending from a few inches to about 4 feet in front of the mouth. While in this position, and within a second of sneezing, the cloud was seen to "clear" by evaporation of the great majority of the droplets, presumably the small droplets which became nuclei of subvisible size. This observation that the majority of droplets become nuclei within one second after leaving the mouth, confirms the calculations of Wells (1934) and the photographic observations of Jennison (1942). When at one second after sneezing the large droplets had fallen to the ground and the numerous small droplets had disappeared by evaporation, there still remained a considerable number of droplets, presumably medium-sized, suspended in the air within 4 feet in front of the mouth. Some of these "medium-sized" droplets fell slowly through 5 feet to reach the ground at times ranging from 1 second to between 5 and 20 seconds after sneezing; a continuous rain of these droplets fell upon the ground usually until about the 12th second after the sneeze. Other "medium-sized" droplets fell slowly through a large, small or negligible part of the distance to the ground and then ceased to fall, presumably having evaporated to nuclei. These presumed nuclei were seen floating in the air until they drifted out of the field of observation; their aerial travel was often watched for over ½ minute.

Section 13: Measurement of droplet projection distance by exposure of paper to dye-containing spray.

The true projection distance of droplet spray, that of the large droplets which do not become nuclei, must be distinguished from the travelling distance of nuclei by airborne drift. This distinction is difficult if a cultural method is employed for demonstration of the droplets, since such a method does not distinguish between droplets and nuclei. In the present investigation a method was used which did not involve culture. A long wide strip of white paper was laid on the floor stretching forwards from the feet of the test subject. The test subject took Congo red into his mouth and then discharged droplet spray. The red marks of the droplets which fell on the paper were clearly visible. Probably even the largest droplet nuclei were too small to be visible on the paper. In any case, the larger marks could be identified with certainty as produced by droplets and not by nuclei. The distance on the floor between the feet of the test subject and the furthest droplet mark was measured.

The maximum projection distance observed was 10½ feet for 'natural sneezes', 8 feet for 'lip coughs', 8½ feet for 'tongue-teeth coughs' and 5 feet for speech. Only a few droplets, the largest, were projected for distances approaching these maxima. The greatest concentration of droplets, mainly of small size, was found on the floor in a region extending from the toes of the test subject to about 4 feet in front.

Section 14: Microscopical observation of morphology of droplets and droplet nuclei.

The morphology of droplets was studied by microscopical observation of the deposit marks which were left on slides after impingement, flattening and evaporation. It was important to know the relationship between the morphology of the droplets and the appearance of their deposit marks. The process of evaporation was watched under the low power objective of the microscope. A slide was held in front of the mouth, bespattered with droplets and at once put on the microscope stage. A large droplet was located and focussed. The droplet was seen always as a low convex bead of hemilenticular shape; this was due to spread and flattening which followed its impingement on the slide. As it evaporated the perimeter of the liquid bead retracted centrally until all liquid had disappeared. The retracting edge of the liquid left behind it on the slide surface a visible deposit of particulate matter, crystals, bacteria, epithelial cells and debris. In all cases, whether on a glass or celluloid surface, the perimeter and area of this deposit mark corresponded exactly with the perimeter and area of the liquid bead before its evaporation. Thus, diameter measurements of droplet deposit marks can be assumed to be equivalent to the diameters of the parent liquid beads at the instant after impingement.

Only in the case of large droplets was the

particulate matter in the deposit mark sufficiently abundant to render its form and outline clearly visible when unstained. The most effective method for demonstrating droplet marks was to colour the mouth secretions with a dye prior to spray production. When 0.1 grams of Congo red had been taken into the mouth, even the smallest droplet marks were colored a deep orange-red and showed sharply demarcated edges (see Figure 1). This was the method of choice when the droplet marks were to be counted or measured.

On the other hand, the internal morphology of droplets was masked by a high concentration of Congo red. For study of the contents of droplets, dye was not taken into the mouth, but instead the droplet marks were stained by a routine bacteriological staining method. The droplet bespattered slide was dried, fixed by flaming and stained by the Gram method or the Ziehl-Neelsen method. By these methods the bacteria and tissue cells were darkly stained and the entire ground substance of the deposit mark was lightly stained; unfortunately, the perimeter of the mark was not always clearly distinguishable. The smaller droplets expelled by healthy persons usually contained nothing more than a little stainable amorphous debris. The larger droplets contained in addition a small or large number of commensal bacteria and sometimes one or more epithelial cells; the bacteria were mostly Gram-negative diplococci and Gram-positive diphtheroid bacilli/

bacilli and streptococci. Commonly the commensal bacteria were concentrated on the surface of the epithelial cells. Figure 2 is a photomicrograph of a normal droplet deposit mark containing one epithelial cell and numerous commensal bacteria. It is to be contrasted with Figure 12, a photomicrograph of the deposit mark of a cough droplet from a tuberculous patient; this latter deposit mark contains tubercle bacilli and polymorph leucocytes.

The morphology of droplet nuclei was studied by coloring the mouth secretions with a dye prior to spray production, collecting the dye-containing nuclei from the air on to a microscope slide, and examining these nuclei with the microscope. Figures 4,5 and 6 are photomicrographs and Figures 7,8 and 9 are drawings of dye-containing droplet nuclei as seen with the oil-immersion objective. When the dye used was Congo red or eosin, their bright red colour distinguished clearly the droplet nuclei from the black dust particles. When India ink was used to colour the oral secretions, the nuclei were black like the dust particles and could be distinguished only by their shape and larger size (see Figure 9). Most of the droplet nuclei were nearly spherical, but some were oval, spindle-shaped or disk-shaped. Usually the nucleus surface was irregular, with rounded indentations, ridges and protuberances. These irregularities of the surface were/

were proof of the solid nature of the nuclei. If the nuclei had been only semisolid, with part of their water still unevaporated, their contours would have been perfectly smooth and circular. Further proof of the three-dimensional solidity of the nuclei was given by their depth of focus. The nuclei obviously were not flattened on the slide. When the edges of the large nuclei were focussed sharply, the small nuclei, dust particles and scratches on the slide surface were out of focus and blurred; when the small nuclei, dust particles and scratches were sharply focussed, the margins of the large nuclei were out of focus and blurred. Figures 5 and 6 are photomicrographs of the same field of nuclei taken at different depths of focus to illustrate these indications of three-dimensional solidity.

When droplets and droplet nuclei were collected together on the same slide, it was an easy matter to distinguish the droplet nuclei from the deposit marks left by the liquid droplets. The three-dimensional solidity and high dye content of the nuclei differentiated them from the flat circular droplet deposit marks of lighter colour (see Frontispiece),

Section 15: Estimation of droplet numbers by method i: counting colonies on blood agar plates exposed directly to spray in front of mouth and nose.

A 12 sq.in. blood agar plate was held at 3 inches from the mouth to receive the spray produced by the expiratory activity being studied. The plate was uncovered by removal of its lid only long enough for this purpose. After incubation the colonies were counted.

When the culture plate was uncovered for only a few seconds, as in the test of a cough or a sneeze, there was little opportunity for accidental contamination by deposition on it of airborne dust particles; in these cases it was assumed that all the colonies found on the plate originated from secretion droplets and the colony count was taken to represent the number of droplets. When the culture plate was uncovered for a longer period, as in tests of speaking or breathing for one-minute periods, it was possible that many of the colonies resulted from contamination with airborne dust particles originating from the clothing of the test subject and from other sources. In control tests, blood agar plates were exposed behind the head of the test subject for one-minute periods; frequently, these gave between 5 and 20 colonies, mostly of Staph.albus, Sarcinae and diphtheroid bacilli. The control plates did not yield Neisseria or alpha haemolytic colonies resembling Strept.viridans (it is to be noted that micrococci giving alpha haemolytic colonies/

colonies resembling Strept.viridans colonies often comprise 1% or 2% of the normal dust-borne bacterial flora of air; see Part 3). In contrast, the colonies obtained on plates inoculated with mouth-spray were mainly Strept.viridans (50%-80%) and commensal Neisseria. The colonies of Strept.viridans were therefore taken as indicative of respiratory tract origin. In the tests in which the culture plates were uncovered for one minute or more, counts were made of the Strept.viridans colonies alone and these counts were taken to represent the numbers of secretion droplets. This procedure must have resulted in the droplet numbers being slightly underestimated, but it obviated the more serious error of gross overestimation by inclusion of the contaminant colonies. In tests of nose breathing this procedure could not be employed, since the nose-spray organisms, Staph.albus and diphtheroid bacilli, were similar to the air organisms.

Table 1 shows the actual colony counts obtained in many experiments with each of 11 different kinds of expiratory activity and also the average (arithmetic mean) count for each activity.

These colony counts underestimate the total droplet numbers since the smaller droplets do not impinge on the plate and many droplets do not contain bacteria capable of growth on blood agar. The proportion of droplets revealed by this method probably ranged from about 5% for sneezing to about 30% for speaking (see Section 30 and Tables 16 and 18).

Section 16: Estimation of droplet numbers by method ii: counting by microscopical observation the deposit marks of dye-containing droplets caught on celluloid slides exposed directly to spray in front of mouth.

A celluloid-surfaced slide was used in preference to a plain glass slide as it was found that the droplets spread less on celluloid and were less likely to become confluent and uncountable. A square piece of celluloid having an area of 1 sq.in. was cut from a cleaned photographic (X-ray) film. This was fixed with balsam to the middle of a 3 in. x 1 in. glass microscope slide. Only the celluloid-surfaced part was scanned for counting of the droplet marks. The celluloid was mounted on the glass slide merely for convenience of manipulation with the mechanical stage of the microscope. About 0.1 grams of Congo red was taken into the mouth of the test subject. The celluloid-surfaced slide was held vertically at 6 inches from the mouth with the celluloid surface directly in front of and facing the mouth. The test subject undertook the expiratory activity being studied and discharged spray at the slide. The droplets were allowed to dry on the slide; they dried rapidly leaving circular and oval red-colored marks. The slide was then observed with the low power of the microscope and scanned methodically in parallel bands by use of the mechanical stage; all droplet marks on the 1 sq.in. piece of celluloid were counted. When, as occasionally happened, part of the celluloid surface

was/

was uncountable because of confluence of the droplet marks, a known fraction of the surface was counted which excluded the uncountable part, and the count was multiplied by the appropriate factor.

The counts obtained by this method represented the number of droplets per square inch of the spray cross-section at 6 inches in front of the mouth. To obtain an approximate estimate of the total number of droplets in the spray, this count per square inch was multiplied by the number of square inches making up the whole area of the spray cross-section in the vertical plane at 6 inches from the mouth. This area of cross-section was measured in several experiments as follows. Congo red was taken into the mouth of the test subject who then discharged spray at a quarto sheet of white paper held vertically at 6 inches in front of the mouth. The sheet of paper was not used intact but after conversion into a grid by cutting out of parallel strips; this minimised deformation and spreading of the spray due to obstruction of the air-stream. As result of a single expiratory act the paper grid became heavily bespattered with easily visible red-colored droplet marks. Most of the marks were concentrated in a roughly circular area in the centre of the grid; this area was measured and taken as equivalent to the spray cross-section. The average area for 6 sneezes was about 20 sq.in., and for 12 coughs about 10 sq.in..

Table/

Table 2 shows the results obtained by this method in 12 tests of each of 5 different kinds of expiratory activity. The numbers given are the actual droplet-mark counts per square inch multiplied by 20 in the cases of sneezes and by 10 in the case of coughs and speaking. They are considered to represent the total number of droplets expelled which were large enough to be caught on the exposed slides, but only as approximations with a low degree of accuracy. The table also shows the average number (arithmetic mean) for each activity.

This method underestimates the true droplet numbers since the smaller droplets do not impinge on the slide. The proportion of droplets revealed by the method probably ranged from about 8% for sneezing to 40% for speaking (see Section 30 and Table 16).

Section 17: Estimation of droplet numbers by method iii: counting colonies on blood agar plates exposed in slit sampler for collection of droplet nuclei from air of chamber contaminated with droplet spray.

The test chamber was allowed to stand closed and undisturbed for at least one day prior to each test so that any previous infection of the air might be eliminated by sedimentation and death of the bacteria. When the 1700 cu.ft. chamber or the 70 cu.ft. chamber was used, an electric desk-type fan was started at the beginning of the experiment and run at half speed until the end of the experiment, blowing diagonally upwards and across the chamber. Before spray production, 1 cubic foot of air was sampled on to a blood agar plate in the slit sampler; this 'control' sample gave a measure of any initial bacterial contamination of the air due either to persistence from the previous experiment or to concurrent raising of dust by the observer or the test subject. The test subject then undertook the expiratory activity being studied and discharged the spray into the chamber. The lapse of half a minute was allowed after this, for the formation of droplet nuclei and their uniform distribution throughout the chamber. During the next minute, that is between ½ and 1½ minutes after spray production, 1 cubic foot of air was sampled on a blood agar plate exposed in the slit sampler. After incubation, the colonies on the 'test' and 'control' plates were counted. The 'control' count was taken to represent the naturally occurring dust-borne air infection and

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the 'test' count to represent the dust-borne air infection plus the air infection produced by the droplet spray. The 'control' count was subtracted from the 'test' count and the difference was multiplied by the number of cubic feet in the chamber to give the total number of droplet nuclei in the chamber, The 'control' samples usually yielded per cubic foot of air some 5 to 10 colonies of Staph.albus, Sarcinae and diphtheroid bacilli, but hardly ever any colonies of Strept.viridans or of alpha haemolytic micrococci. Most of the colonies on the 'test' plates were of Strept.viridans. In tests of speaking and coughing the 'test' count was often not much greater than the 'control' count; estimates computed from the difference of these counts would have been subject to large errors due to random sampling variation in the two counts. To avoid such errors, use was made of Strept.viridans as an indicator of droplet spray origin. When the 'test' count was less than 40 per cubic foot, a count was made of the Strept.viridans colonies alone and was multiplied by the number of cubic feet in the chamber to give the total number of droplet nuclei.

Table 3 shows the results obtained by this method in 9 to 23 tests of each of 9 different kinds of expiratory activity. The numbers shown are the actual colony counts per air sample multiplied by the ratio of chamber volume to sample volume; they are considered

to/

to represent the total number of droplet nuclei containing viable bacteria which were produced on each occasion. The average number (arithmetic mean) is also given for each activity.

This method underestimates the true droplet numbers since it does not reveal the droplets which are too large to become nuclei and the droplets which do not contain viable commensal bacteria. The proportion of droplets revealed by the method was probably between 5% and 10% (see Section 30 and Tables 16 and 18).

Section 18: Estimation of droplet numbers by method iv: counting by microscopical observation the dye-containing droplet nuclei on oiled slides exposed in slit sampler for collection of nuclei from air of chamber contaminated with droplet spray.

The test chamber was left closed and undisturbed for at least one day prior to each experiment. When the 1700 cu.ft. chamber or the 70 cu.ft. chamber was used, an electric fan was run at half speed throughout the experiment. Before spray production, a 'control' sample of 1 cubic foot of air was taken on to an oiled slide in the slit sampler; this was required in order to prove that there were not any red-colored particles in the air prior to its contamination by droplet spray. The test subject took Congo red into his mouth and discharged droplet spray into the chamber by undertaking the expiratory activity to be studied. Half a minute was allowed for formation of the droplet nuclei and their uniform distribution throughout the chamber. During the next minute, that is between ½ and 1½ minutes after spray production, 1 cubic foot of air was sampled on to an oiled slide in the slit sampler. If the droplet nuclei were very numerous, only a ¼ or ½ cubic foot of air was sampled. The oiled slides which were used instead of culture plates in the slit sampler, were prepared by spreading glass microscope slides very thinly with a 5% solution of boiled linseed oil in chloroform and allowing them to dry. The slide was placed on the platform of the slit sampler with its oiled surface at 2 millimeters below the/

the air intake slit. The platform was not rotated and thus the air dust and droplet nuclei were deposited in a thin, easily visible line. This "dust-line" was of uniform density throughout its length. It was 29 millimeters long. Its width was indefinite, for although most of the particles were concentrated in a central strip a ½ millimeter in width, a few were scattered on either side of this for distances of up to 1 millimeter. A drop of immersion oil was placed on the slide over and in direct contact with the dust-line. The dust particles and droplet nuclei rarely were disturbed or floated off the slide surface by the oil. The dust-line was examined with a microscope using a mechanical stage, an oil-immersion (1/12 in.) objective and a (x 8) eyepiece incorporating a micrometer scale. The scale contained 10 major and 100 minor divisions; its whole length represented 170 microns of the dust-line. In searching for droplet nuclei, the dust-line was scanned in transverse bands of a width corresponding to the length of the micrometer scale; that is, each band was 170 microns of the length of the dust-line. The scanning was carried out by setting the micrometer scale parallel to the dust-line and moving the slide so that the dust-line passed from side to side under the scale (Figures 3,5,6). Their red colour made the Congo red-containing nuclei readily distinguishable from the black dust particles (Figures 4,7). The search for nuclei was continued in transverse/

transverse bands selected at intervals along the length of the dust-line until a significant number had been counted, usually between 300 and 500. If the nuclei were scanty, the whole dust-line might have to be searched before even a few dozen nuclei were found. If the nuclei were moderately numerous, only 10, 20 or 30 transverse bands of 170 microns width were scanned. If the nuclei were very numerous, narrower transverse bands were examined, bands covered by the two central major divisions of the scale and representing 34 microns of the length of the dust-line. Appropriate multiplication of the counts gave the number of nuclei in the whole dust line (i.e. in $\frac{1}{4}$, $\frac{1}{2}$ or 1 cubic foot of air) and further multiplication gave the number of nuclei in the total volume of the chamber. As the initial counts were subject to the usual error of random sampling, and as the calculation involved a big multiplication (e.g. by 3500 for a sneeze and by 10 for a cough in tests using the 70 cu.ft. chamber), the figures obtained for the total number of nuclei were only rough approximations to the true nucleus numbers.

Table 4 shows the results obtained by this method in 16 to 20 tests of each of 6 kinds of expiratory activity. The numbers shown are the actual counts multiplied by the appropriate factor to give the total number of nuclei in the test chamber. They represent the/

the total number of droplet nuclei produced, and thus the total number of droplets small enough to become nuclei. The average number (arithmetic mean) is given for each activity.

The method slightly underestimates the true droplet numbers since it does not reveal droplets which are too large to become nuclei. Moreover, it is possible that there are nuclei smaller than the limit of microscopical resolution which also would be missed. Neglecting this latter possibility, it was calculated that the proportion of droplets revealed by the method ranged from about 75% for speaking to about 97% for sneezing (see Section 30 and Table 16).

Section 19: Counting droplet nuclei by the cultural method (iii) and the microscopical method (iv) employed in parallel.

The presence of Congo red did not seem to affect the viability of bacteria contained in droplet nuclei. The colony counts given by Congo red-containing spray were just as great as those given by plain secretion spray. Accordingly, in many of the experiments with Congo red, the cultural method of counting was employed in addition to the microscopical. The air was examined alternately by the two different methods. An air sample was taken on to a blood agar plate immediately after the taking of each sample on to an oiled slide. The same slit sampler was used to take both samples. The slide was exposed for ¼, ½ or 1 minute. Removal of the slide and substitution of the culture plate occupied a ½ minute. The culture plate was exposed for 1 minute. This procedure made possible direct comparison of the efficiencies of the two methods and revealed the proportion of the droplet nuclei which contained viable bacteria.

Tables 30-37 show for 8 tests of a 'natural sneeze' the numbers of microscopically visible nuclei (second last column) and the corresponding numbers of nuclei containing viable bacteria (last column). The bacteria-carrying nuclei comprised only a small proportion of the microscopically visible nuclei (e.g. for the ½-1 minute counts, 3.2%, 7.3%, 2.5%, 1.2%, 8.3%, 12.4%, 3.9% and 7.3%; on average, 5.8%).

Section 20: Differential counting of droplets originating from the throat and droplets originating from the nose.

The majority of secretion droplets undoubtedly originate from the anterior part of the mouth. A few may originate from the throat and a few from the nose. Special experiments were made to count separately the droplets originating from the throat and the droplets originating from the nose. One method used for counting the nose droplets was to observe the droplet spray discharged while the mouth was covered by an impermeable mask; the counts thus obtained in 15 tests of a 'natural sneeze' are given in Table 1.

A second method enabled differential counting of either the throat droplets or the nose droplets; this involved use of B.prodigiosus (Ser.marcescens) as an indicator of the site of origin of the droplets. B.prodigiosus is non-pathogenic and heavy inoculation into the respiratory tract is without danger. This bacterium does not normally reside as a commensal in any part of the respiratory tract; for this reason it can only be present in a droplet if the droplet originates from a locality which has been inoculated artificially with a culture. The red colonies of B.prodigiosus are distinctive; they are easily recognised and easily counted.

A highly pigmented strain of B.prodigiosus was obtained by selective subculture of an old stock culture./

culture. A cotton wool swab was rubbed in a 24-hour nutrient agar culture of this B.prodigiosus strain and was then applied to the throat or nose. For investigation of throat origin, the tonsillar regions, the free edge of the soft palate and the back of the tongue were inoculated; the anterior mouth was then proved free of B.prodigiosus by culture of a swab taken from the front teeth, lips and tip of tongue. For investigation of nasal origin, the anterior nares and forward parts of the nasal cavity were inoculated, while B.prodigiosus was not put into the mouth or throat. Immediately after inoculation with B.prodigiosus, the test subject undertook the expiratory activity being studied. The spray was examined by one of two methods. In some tests the spray was caught on 12 sq.in. nutrient agar plates held at 3 inches in front of the mouth and nose. In other tests the spray was introduced into the 2½ cu.ft. chamber and the air of this was examined for droplet nuclei by use of the slit sampler with nutrient agar plates. After incubation of the plates, the B.prodigiosus colonies on each were counted.

Table 5 shows the B.prodigiosus colony counts obtained on plates exposed directly to spray in front of the mouth and nose in 15 to 30 tests of each kind of expiratory activity. The numbers given represent the/

the larger droplets of throat-origin only or the larger droplets of nose-origin only. The average number (arithmetic mean) for each activity is also given.

Table 6 gives for 10 tests of each kind of expiratory activity the B.prodigiosus colony counts made on plates exposed in the slit sampler multiplied by the ratio of chamber volume to sample volume; these numbers represent the droplets of throat-origin only or the droplets of nose-origin only which were small enough to become nuclei. The average number (arithmetic mean) for each activity is also given.

Section 21: Micrometric measurement of deposit marks of dye-containing droplets caught on celluloid slides exposed directly to spray in front of mouth.

The test subject took Congo red into his mouth and discharged droplet spray at a celluloid-surfaced microscope slide held vertically at 3 or 6 inches in front of the mouth, 3 inches in tests of speaking and 6 inches in tests of coughing and sneezing. After drying of the droplets, the slide was scanned with the low power (2/3 in.) objective of the microscope using a mechanical stage. The diameters of the first few hundred droplet marks encountered on the slide were measured by use of an eyepiece with a micrometer scale. The droplet deposit marks (see Figure 1) were easily visible because of their red colour, clearly delimited and mostly circular. A few marks were oval or pear-shaped; the mean diameter of each of these was calculated from the measurements of its minimum and maximum diameters.

Table 9 (with reference to the first column) shows the measurements of 3000 droplet marks produced by each of 5 different kinds of expiratory activity. In all, 74 slides which were exposed to separate expiratory acts were examined to obtain these results. The number of droplets in each size group is shown. Table 10 (with reference to the first column) shows the same results expressed as the percentages of droplets occurring in each size group.

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The error of micrometric measurement was small. Measurement was certainly accurate to the nearest quarter of a small division of the micrometer scale, that is to the nearest 4 microns when the low power (2/3 in.) objective was used. Thus the maximum possible error must have been less than 20% for a 20 microns droplet mark and less than 0.4% for a 1000 micron droplet mark; most of the droplet marks lay between these limits.

A further possible cause of error in this method for estimating droplet size is that the droplets may have become reduced in size by evaporation during the time of their flight from the mouth to the slide. Assuming the projection velocities were between 10 and 100 feet per second (see Jennison, 1942), the time of travelling 6 inches from mouth to slide would be between 0.05 and 0.005 seconds. The evaporation times of water droplets in unsaturated still air at 22°C., as given by Jennison (1942), are 0.02 seconds for a droplet of 12 microns diameter, 0.08 seconds for a 25 micron droplet and 0.31 seconds for a 50 micron droplet. Saliva droplets in moisture-saturated breath would probably evaporate more slowly; however, even with evaporation times as short as these, only droplets of 25 microns diameter or smaller would diminish appreciably in size before reaching the slide.

Section 22: Calculation of diameters of spherical parent droplets from measured diameters of droplet deposit marks.

In order that the sizes of the spherical parent droplets might be calculated from these measurements of dried droplet deposit marks on celluloid slides, special experiments were made to discover how much droplets become flattened when impinging on a slide; the ratio between spherical parent droplet diameter and deposit mark diameter was determined. These experiments were made according to the method of Strausz (1926). Drops of saliva were delivered from the point of a fine capillary tube clamped opposite the low power (2/3 in.) objective of a horizontally placed microscope. Drops of different size, between 1 and 3 millimeters in diameter, were obtained by use of differently sized tubes. The diameter of each droplet while hanging in a nearly spherical shape from the point of the capillary tube, was measured by use of the eyepiece micrometer scale. The droplet was then allowed to fall about 4 inches on to a microscope slide, sometimes glass and sometimes celluloid-surfaced. After it had dried on the slide the droplet's deposit mark was measured micrometrically.

Table 8 shows the measurements obtained in these experiments. Droplets of saliva falling on glass slides were found to spread to 3 times their original diameter. This finding accords with the findings of Strausz (1926). Accordingly, when glass slides were/

were used, the diameter of the spherical parent droplet was calculated as one third of the measured diameter of the droplet deposit mark. In contrast, droplets falling on celluloid-surfaced slides were found to spread to only about 2 times their original diameter. When celluloid slides were used, as for the measurements given in Tables 9 and 10, the diameter of the spherical parent droplet was calculated as half of the measured diameter of the droplet deposit mark.

It is of interest to know the possible extent of error involved in calculating the diameters of parent droplets as half the diameters of the droplet marks on celluloid slides. Of the observations recorded in Table 8, 1 to $1\frac{3}{4}$ and 1 to $2\frac{1}{2}$ were the ratios differing most from the 1 to 2 ratio. Almost certainly the true ratios were between 1 to $1\frac{1}{2}$ and 1 to 3; if so, the maximum error in the calculated parent droplet diameter was less than 1.5-fold (i.e. plus 50% or minus 33%).

Tables 9, 10 and 14, with reference to the right hand column, and Graph 1 show <u>in terms of the calculated diameters of the spherical parent droplets</u>, the size-distribution of the droplets which were caught on celluloid slides exposed in front of the mouth.

Section 23: Micrometric measurement of dye-containing droplet nuclei collected from air of a chamber contaminated with droplet spray on to oiled slides exposed in slit sampler.

The test subject took about 0.1 grams of Congo red into his mouth and then undertaking the expiratory activity being studied discharged droplet spray into one of the test chambers. Air samples were taken by the slit sampler on to oiled slides. The dust-line on each slide was scanned with the microscope using an oil-immersion (1/12 in.) objective, a (x 8) eyepiece with a micrometer scale and a mechanical stage. The diameter was measured of each of the first few hundred nuclei encountered as the dust-line was scanned systematically in transverse bands passed beneath the micrometer scale (Figures 3,5,6). According to their diameters the nuclei were allocated to the different size classes shown in the table of results (Table 11). The smallest droplet nuclei distinguished were near to the limit of resolution of the microscope; these were classed as ¼ to ½ micron in diameter, but measurement could not be very accurate at such a small size.

A total of more than 25,000 droplet nuclei were measured in 113 tests of expiratory acts of 7 different kinds. The nucleus diameters observed in certain individual experiments are shown in Tables 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41 and 42. Table 11 shows the measurements for all experiments taken together; it gives the number of nuclei observed which/

which belonged to each size group. Table 12 shows these same aggregate results expressed as the percentages of nuclei observed which belonged to each size group.

The tests made in the 1700 cu.ft. chamber and the tests made in the 70 cu.ft. chamber yielded similar size-distributions; the results for these two chambers therefore have not been given separately. The similarity of size-distributions was to be expected since the potential falling height for the droplets was the same, 5 feet, in the 1700 cu.ft. chamber and in the 70 cu.ft. chamber. In the 2½ cu.ft. chamber, on the other hand, the potential falling height and the potential projection distance were only 1½ feet, so that there must have been less time available for evaporation and nucleus formation; in consequence, the maximum size of droplet capable of becoming a nucleus must have been less than in the larger chambers. This is probably the reason why relatively fewer large nuclei were found in the 2½ cu.ft. chamber (see Tables 11 and 12). In contrast, relatively more of the very small nuclei were found in the 2½ cu.ft. chamber because they were more easily recognised amid the much less abundant dust (see Tables 11 and 12).

Micrometric measurement with the oil-immersion (1/12 in.) objective were accurate at least to the nearest quarter scale division, that is to the nearest 0.4 microns.

Section 24: Micrometric measurement of dye-containing droplet nuclei collected on to exposed slides by sedimentation from air of chamber contaminated with droplet spray.

For comparison with the droplet nucleus measurements obtained by the above method using the slit sampler, some measurements were also made of droplet nuclei recovered from the air by their simple sedimentation on to slides lying on the floor. The test subject took into his mouth 0.1 grams of Congo red or 0.1 or 0.01 grams of eosin. He delivered one or more simulated sneezes from standing height, with the mouth 5 feet above the floor, either in the 1700 cu.ft. chamber or in the 100 cu.ft. chamber. One minute was allowed after sneezing for all droplets to sediment from the air (except in one experiment). Some unoiled glass slides were then exposed on the floor 2 to 3 feet in front of the test subject and were left thus for 1 or 18 hours. The slides were searched for nuclei under the low and high powers of the microscope; the nuclei encountered were measured micrometrically using the oil-immersion (1/12 in.) objective.

Table 47 shows the measurements of droplet nuclei thus collected by sedimentation. These measurements may be compared with the measurements of droplet nuclei collected by the slit sampler which are shown in Tables 11 and 12. The important difference between the two series of measurements is that a relatively low proportion of small nuclei, under 4 microns, were collected/

collected by sedimentation. Nuclei under 4 microns were not collected in two experiments with the slides exposed to sedimentation during only 1 hour. A considerable number of nuclei from ½ to 4 microns in diameter were collected in two experiments with the slides exposed to sedimentation during 18 hours, but not relatively as many as could be collected by the slit sampler. The small nuclei sediment much more slowly than the large nuclei and it is not surprising that a relatively smaller number settle on to exposed slides.

The largest of the Congo red-containing nuclei collected by sedimentation (35 microns in diameter) were of the same order of size as the largest Congo red-containing nuclei collected by the slit sampler (42 microns in diameter). Without their large Congo red content these nuclei would have been 20% to 35% smaller in diameter. The largest nucleus collected by sedimentation when 0.01 grams of eosin was used to colour the mouth secretions, was 22 microns; in this case the eosin content was so small that without it the nucleus would not have been appreciably smaller.

Section 25: Estimation of diameters of unevaporated parent droplets from measured diameters of dye-containing droplet nuclei.

In order to calculate the original size of the parent droplets from the measurements of droplet nuclei, it was necessary to know how much shrinkage occurred as a result of evaporation. Measurements of droplets before and after evaporation, and calculations from the concentration of dissolved and suspended solid matter in saliva, both indicated that the ratio between droplet nucleus diameter and parent droplet diameter was about 1/4 for Congo red-containing saliva and 1/5 for plain saliva. These ratios were accepted for calculation of parent droplet diameter.

Special experiments were made in which large saliva droplets were measured before evaporation and their residues, droplet nuclei, measured after evaporation. A sample of saliva was withdrawn from the mouth of the test subject half a minute after introduction of the usual quantity, about 0.1 grams, of Congo red. Drops of this dye-containing saliva were suspended from the ends of fine glass fibres. The initial diameter of the droplet was measured micrometrically and then, after evaporation, the final diameter of the residual droplet nucleus. It was important that the glass fibre should be as thin as possible in relation to the size of the droplet so as to avoid exaggeration of the size of the droplet or droplet nucleus due to inclusion of an appreciable

volume of glass. The best which could be achieved was the suspension of droplets about 1 millimeter in diameter from glass wool fibres about 0.015 millimeters in diameter; larger droplets could not be caught and retained on the end of the fibre. To aid retention of the droplet, the end of the glass fibre was very slightly expanded by flaming. The fibre was stuck to a strip of wood which was clamped rigidly to a retort stand; the free end of the fibre projected vertically downwards from the wood. The drop of saliva was extruded from the end of a fine capillary pipette on to the upper part of the fibre. On becoming free from the pipette the drop slid down the fibre to the free end where it remained suspended. The hanging drop was at once viewed with the low power (2/3 in.) objective of a horizontally placed microscope; the diameter of the drop was measured by use of the eyepiece micrometer scale. The drop was then left undisturbed until its evaporation was complete and it showed no further shrinkage in observations repeated at intervals. The dried residue, that is the droplet nucleus, was sometimes nearly spherical and sometimes markedly aspherical. If it was nearly spherical its diameter was measured micrometrically.

Table 13 shows the parent droplet diameter ('initial') and the droplet nucleus diameter ('final') for 12 Congo red-containing saliva droplets and for 11 plain saliva droplets. The ratio of droplet nucleus/

nucleus diameter to parent droplet diameter ranged from 1/3 to 1/6, on average 1/4, for Congo red-containing saliva, and ranged from 1/3½ to 1/7½, on average 1/5, for plain saliva. As was to be expected, the presence of Congo red in the saliva appreciably increased the size of the nucleus in relation to the size of the parent droplet, giving an average diameter ratio of 1/4 as compared to 1/5.

Calculation from the concentration of dissolved and suspended solids in Congo red-containing and in plain saliva indicated similar ratios between droplet nucleus diameter and parent droplet diameter. The irregular, 'solid' appearance of droplet nuclei observed microscopically was evidence that nearly all water was lost in droplet nucleus formation. The specific gravities of saliva and saliva solids was assumed not to differ greatly from the specific gravity of water. Thus, the ratio of the droplet nucleus diameter to the parent droplet diameter, which is equal to the ratio of the cube root of the droplet nucleus volume to the cube root of the droplet volume, was considered to be equal to the ratio of the cube root of the number of grams of solids in 100 grams of saliva to the cube root of 100. Accordingly, the ratio of droplet nucleus diameter to parent droplet diameter would be 1/3 if the solid content of the saliva was 3.7%, the ratio 1/4 if the solid content 1.6%, the ratio/

ratio 1/5 if the solid content 0.8%, the ratio 1/6 if the solid content 0.46%, and the ratio 1/7 if the solid content 0.3%. The natural solid content of saliva usually is between 0.3% and 1.5%; thus, for plain saliva spray, the ratio of droplet nucleus diameter to parent droplet diameter will be between 1/7 and 1/4. When 0.01 grams of eosin was taken into the mouth, the solid content of the saliva probably was not raised more than 0.2% to 0.4%, so that the ratio of droplet nucleus diameter to parent droplet diameter would not be much greater than for plain saliva. When 0.1 grams of Congo red was taken into the mouth, the additional concentration of Congo red, as estimated colorimetrically, varied between 1% and 4%, being usually about 2%. Thus, the total solid content of Congo red-containing saliva usually must have been between 2% and 4%, and the ratio of droplet nucleus diameter to parent droplet diameter between 1/4 and 1/3. Congo red-containing saliva expectorated in four experiments was pooled and evaporated on a water bath; by weighing, its total solid content was found to be 1.8%, indicating a nucleus-parent droplet ratio of 1/3.

It was concluded that the diameters of parent droplets before evaporation could best be estimated as four times the measured diameters of the Congo red-containing droplet nuclei. The greatest error possible in this calculation seems to be that the true nucleus-parent droplet ratio might be 1/3 instead of 1/4/

1/4; in this case, 33% would be the maximum error in the estimated droplet diameter.

Table 14, with reference to the right hand column, and Graph 2 show in terms of the calculated diameters of the parent droplets, the size-distribution of the droplets which became droplet nuclei and were collected as such from the air in the experiments recorded in Table 11.

Section 26: Construction of a composite size-distribution series for the droplets of all sizes expelled in speaking, in coughing and in sneezing.

Of the two methods employed for collection and measurement of droplets, one, collection on slides exposed in front of the mouth, demonstrated only the large and medium sized droplets (over 20 microns in diameter), while the other, collection of droplet nuclei by the slit sampler, demonstrated only the small and medium sized droplets (under 100 microns in diameter). To obtain a comprehensive size-distribution series covering all the droplets, large, medium and small, it was necessary to combine the two size-distribution series given, respectively, by measurement of droplets collected on slides exposed in front of the mouth (Table 9; Graph 1) and by measurement of droplet nuclei collected with the slit sampler (Table 11; Graph 2). The two series had to be jointed at some point in the size range of the medium sized droplets (20-100 microns in diameter) at which both methods of collection were efficient.

To enable construction of composite droplet size-distribution series for each of the three activities, "speaking", "coughing" and "sneezing", the basic data were assembled in Table 14. The size-distributions of droplets caught on slides exposed in front of the mouth were taken from Table 9 and the size-distributions of droplets collected as nuclei by the slit sampler were taken from Table 11. The figures in/

in Tables 9 and 11 for "Speaking Loudly" were placed in Table 14 for "Speaking". The figures in Tables 9 and 11 for "Throat-only Coughs", "Tongue-teeth Coughs" and "Lip Coughs" were added together to form a single series which was placed in Table 14 for "Coughing". The figures in Tables 9 and 11 for "Natural Sneezes" and "Simulated Sneezes" were added together to form a single series which was placed in Table 14 for "Sneezing". In Table 14 the numbers of droplets belonging to each size group are expressed as percentages of the total numbers of all sizes. The size groups are designated both in terms of the original measurements, that is of droplet nucleus diameter or droplet deposit mark diameter, and in terms of the calculated diameters of the parent droplets. The diameters of the parent droplets were calculated, as explained in Sections 22 and 25, as 4 times the measured diameters of the droplet nuclei and as half the measured diameters of the droplet deposit marks on celluloid slides.

The composite droplet size-distribution was calculated on the basis of the observed distributions as presented in Table 14. The main problem was to decide at what point to joint the size-distribution series for the larger droplets (lower half of Table 14; from Table 9) with the size-distribution series for the smaller droplets (upper half of Table 14; from Table 11).

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It was obvious that the two series must be joined somewhere in the range of the medium sized droplets, between 20 and 100 microns diameter. To help decision as to the exact point of junction, size-distribution curves were drawn from the figures in Table 14 in terms of the calculated diameters of the parent droplets: Graph 3 for speech droplets, Graph 4 for cough droplets and Graph 5 for sneeze droplets. Each graph shows two size-distribution curves, one given by each of the two methods of collection. In the main, the curves for speech droplets, for cough droplets and for sneeze droplets are similar. In each case, starting from the upper droplet size range, the curves given by the two methods of collection both show a rise in droplet numbers with decrease in droplet size; in fact, the smaller droplets were more numerous than the larger. The size-distribution curve of the droplets collected as nuclei by the slit sampler continues to rise until a maximum point is reached between the 5 micron and the 10 micron abscissae; the commonest droplet diameters were between 5 and 10 microns. In contrast, the size-distribution curve of the droplets collected on slides exposed in front of the mouth begins to flatten out about the 30 micron abscissa and is falling rapidly at the 20 micron abscissa; this is obviously due to most of the droplets under 20 microns in diameter and some of the droplets between 20 and 30 microns failing to be caught on/

on the slides exposed to spray in front of the mouth. The size-distribution of droplets as revealed by measurements of droplet marks on the exposed slides is defective in the diameter range below 30 microns.

Droplet nuclei of up to 42 microns in diameter were collected by the slit sampler, but nuclei larger than 25 microns were very rare (e.g. only 4 out of 25,175 in Table 11) and nuclei larger than 12 or 14 microns were absent in many of the individual experiments contributing to the aggregate results shown in Table 14. For this reason, the size-distribution of droplets as revealed by measurements of nuclei collected by the slit sampler is regarded as probably defective in the droplet diameter range above 50 microns (i.e. for nucleus diameters above $12\frac{1}{2}$ microns).

The range from 30 to 50 microns in parent droplet diameter thus appeared to be the only size range in which the size-distribution series given by the two methods of collection were both reliable. This 30 to 50 microns "equivalence range" enabled jointing of the two series. It was decided to joint the two series at 40 microns. It was necessary to adapt the series so that their size-distribution curves met at the 40 micron abscissa. Graphs 3, 4 and 5 revealed that the curve for droplets caught on slides exposed in front of the mouth was higher at the 40 micron abscissa than/

than the curve for droplets collected as nuclei by the slit sampler. This ratio of droplet numbers (as percentages per 1-micron range of diameters) at 40 microns was 1 to 1.8 for speech droplets, 1 to 3 for cough droplets and 1 to 12 for sneeze droplets. In order to render the two size-distribution series comparable for junction at 40 microns, it was necessary to multiply one series by the ratio between the numbers per 1-micron range at 40 microns so that in both series there would be the same numbers per 1-micron range at 40 microns.

The composite size-distribution series was constructed as follows. For the droplet diameter groups under 40 microns the percentage figures were taken without alteration from the upper half of Table 14, that is as calculated from droplet nucleus measurements. For the droplet diameter groups over 40 microns the percentage figures from the lower half of Table 14, that is as calculated from droplet deposit mark measurements, were taken after division by the appropriate factor, 1.8 for speaking, 3 for coughing and 12 for sneezing. The figures in each size group were then multiplied by 100 and divided by the total of the figures for all groups in this composite series in order to reduce the group figures in the composite series to the form of percentages.

Table 15 shows separately for speech droplets, for/

for cough droplets and for sneeze droplets the calculated composite size-distribution series in terms of the percentages in each size group of the total number of droplets of all sizes. Table 17 shows the same series, but giving instead of percentages the absolute numbers of droplets in each size group out of a total of 100 droplets for speaking 100 words, 1000 droplets for a cough and 1,000,000 droplets for a sneeze; these totals were selected as being the approximate average numbers expelled by the expiratory activities in question as revealed by the best droplet counting method, namely microscopical counting of nuclei collected by the slit sampler (see Table 4).

These calculated composite droplet size-distribution series shown in Tables 15 and 17 should be regarded as corresponding only approximately to the true size-distributions. Two causes of error were inherent in their calculation. In the first place there was the possible error of 30% or so in the calculation of parent droplet diameter from droplet nucleus diameter or droplet mark diameter. In the second place there was the possibility of overestimating or underestimating the number of large droplets in relation to the number of small droplets by jointing the two size-distribution series incorrectly. As described above the 40-micron abscissa was chosen for jointing and the series were equated by dividing the figures/

figures in the large droplet series by 1.8, 3 and 12, respectively, for speech droplets, cough droplets and sneeze droplets. There was no reason, however, why the two series should not have been jointed at the 30-micron abscissa or the 50-micron abscissa; the division factors would have been 1.2 or 5 instead of 1.8, 1.6 or 6 instead of 3, and 6 or 18 instead of 12. Thus, in calculating the composite series, the number of large droplets in relation to the number of small droplets may have been overestimated by 50-200% (i.e. by 1½-fold to 3-fold) or underestimated by 30-50% (i.e. by 1½-fold to 2-fold).

Even with inaccuracies of the above order, the calculated composite size-distributions shown in Tables 15 and 17 are of value because they give an overall account of droplet spray which is not attainable by any other method.

Section 27: Calculation of the proportion of droplets in each size group which will contain commensal or pathogenic micro-organisms.

The sanitary significance of droplet spray depends not on the total number of droplets expelled, but on the number of infected droplets expelled, the number of droplets containing one or more viable micro-organisms of some particular species. The probability of one or more micro-organisms being contained in a droplet is related in direct linear proportion to the volume of the droplet and in direct linear proportion to the concentration of "viable microbial units" in the secretion atomised. When a number of micro-organisms are firmly joined together in a chain or cluster, or are firmly adherent to an epithelial cell or a food particle, the entire aggregate must come to be contained in the one droplet; this aggregate of micro-organisms is thus equivalent only to a single free micro-organism in influencing the chance of a droplet being infected. The term, "concentration of viable microbial units", is used to mean the number of viable free micro-organisms plus the number of viable aggregates of micro-organisms present in a unit volume of the secretion.

The probability that a droplet in a given size group will contain micro-organisms may be expressed as the proportion of droplets in that size group which will contain micro-organisms. The proportion of droplets/

droplets infected was calculated for each different size group of the composite size-distribution series shown in Table 15. In the first place, it was necessary to decide what concentration of "viable microbial units" should be taken as a basis for the calculations. Fortunately, the usual method for counting viable bacteria in suspensions, the counting of colonies on culture plates inoculated with a known volume of suspension, gives counts in terms of concentration of "viable bacterial units" (viable free bacteria plus viable aggregates). The only available figures for concentration of pathogenic bacteria in saliva were those of Hamburger (1944) for Strept.pyogenes. Hamburger found by culture plate colony counts that the saliva of persons with throat infection contained usually between 1000 and 1000,000 'Strept.pyogenes' per milliliter. The figures, 1000, 30,000 and 1000,000 per milliliter were selected for calculation of the proportion of droplets containing pathogenic organisms. For calculation of the proportion of droplets containing commensal organisms, the figure of 30,000,000 per milliliter was selected. Gordon (1904) examined 25 samples of saliva and found that the commonest commensal, Strept.viridans, was present in numbers varying from 10,000,000 to 100,000,000 per milliliter. In the present study, two estimations were made of the number of viable bacteria of all species present in the saliva of the test subject/

subject (A). Blood agar plates were inoculated with a measured volume of various dilutions of the saliva and, after aerobic incubation, the colonies on each plate were counted. On the two occasions, the saliva was found to contain 23,000,000 and 34,000,000 'bacteria' per milliliter.

The proportion of infected droplets in each size group was calculated as follows. It was assumed that over each size range shown in Table 15 the droplets were distributed equally by diameter. The mean volume of the droplets in each size group was calculated as $\pi/6.\frac{1}{4}(b^4 - a^4)/(b - a)$, where a and b were the minimum and maximum diameters of the droplets in the group. For each size group the mean number of "viable microbial units" present in a droplet of the mean volume was calculated for 30,000,000, 1000,000, 30,000 and 1000 "viable microbial units" per milliliter. The percentage of droplets in each size group which would contain one or more "viable microbial units" was calculated as $100(1 - e^{-m})$, where m was the mean number of "viable microbial units" per droplet in the group and e was 2.718; this calculation was based on the assumption that there was a Poisson type distribution of the "viable microbial units" among the droplets. Table 18 shows these calculated percentages of the droplets in each size group which would contain one or more micro-organisms.

Section 28: Counting by microscopical observation the commensal bacteria in droplets of each size.

Glass microscope slides were exposed vertically at 6 inches in front of the mouth of the test subject (A) to catch the droplets expelled by simulated sneezes. The sprayed slides were fixed by heat and stained by Gram's method with prolonged application of the basic fuchsin counterstain. Each slide was scanned for droplet marks with the low power (2/3 in.) and high power (1/6 in.) objectives of the microscope. The diameter of each clearly delimited droplet mark was measured by use of the eyepiece micrometer scale. The measured droplet mark was then covered with a drop of immersion oil and was examined with the oil-immersion (1/12 in.) objective. All Gram-positive and Gram-negative bacteria found in the droplet mark were counted. Congo red was not taken into the mouth of the test subject in these experiments, since it would have been washed out of the droplet marks in the process of Gram-staining. As they were not colored with Congo red, the droplet marks often showed indistinct outlines. The intensely staining bacteria and epithelial cells were found mainly towards the centre of the droplet mark, while the remainder of the mark was visible only by virtue of the weakly staining granular material (see Figure 2). It is possible that in some cases the true diameter of the droplet mark was larger than the diameter of the visibly stained part/

part which was measured, but it is thought unlikely that great error was incurred in this way.

Table 20 shows the results obtained on examination of 256 droplet deposit marks; it shows the number of bacteria found in each droplet mark and the average number of bacteria per droplet in each size group. The limits of each size group is indicated not only as the droplet mark diameters but also as the spherical parent droplet diameters calculated as one third of the droplet mark diameters. The mean volume of the spherical parent droplets in each size group was calculated as $\pi/6.\frac{1}{4}(b^4 - a^4)/(b - a)$, where a and b were the minimum and maximum diameters of the size group. The average number of bacteria per milliliter of saliva was calculated for each size group on the basis of the calculated mean volume; as was to be expected, the calculated average number of bacteria per milliliter of saliva was similar for the different size groups, mainly about 3000,000,000, and varying only from 800,000,000 to 4400,000,000 (see right-hand column of Table 20). The average number of bacteria per milliliter of saliva should, of course, be the same whatever the size of droplets examined. This constancy of the numbers calculated from droplet measurements and bacterial counts, is to some extent a confirmation of the accuracy of the measuring, counting and computing.

The present microscopical counting of bacteria in measured/

measured droplet marks revealed that the saliva of test subject A contained a total of about 3000,000,000 viable and non-viable bacteria per milliliter. For comparison, it may be recalled that culture plate colony counting, described in Section 27, revealed that the saliva of test subject A contained only about 30,000,000 "viable bacteria units" (viable free bacteria plus viable bacterial aggregates) per milliliter. Non-viability and aggregation of the salivary bacteria was thus responsible for a 100-fold difference between the microscopical and cultural counts.

Section 29: Calculation of the proportion of droplets and nuclei of all sizes which will contain commensal or pathogenic organisms

The proportion of droplets, of all sizes taken together, which will contain commensal or pathogenic micro-organisms, was readily calculated from the known proportions of all droplets which belong to each size group (Table 15) and the known proportions of droplets in each size group which will contain organisms (Table 18). For each size group the latter percentage was extracted from the former and the products so obtained were added together to give a total for all size groups, namely the overall infected percentage of all droplets. A similar calculation made for only the size groups of under 100 microns droplet diameter, gave the overall infected percentage of all droplet nuclei. These calculations were made separately for each of the different salivary concentrations of "viable microbial units", and separately for speech droplets, for cough droplets and for sneeze droplets, these having different size-distributions (see Table 15).

The results of the calculations are shown in Table 19. The organism-containing droplets and nuclei are shown both as percentages of the total number of droplets and nuclei of all sizes, and as actual numbers out of a total of 100 droplets for 'Speaking Loudly 100 Words', out of 1000 droplets for a 'Cough' and out of 1000,000 droplets for a 'Sneeze'. The proportion of droplets and nuclei containing organisms was highest for speaking and lowest for sneezing.

Section 30: Estimation of the efficiency of the different droplet counting methods by consideration of the observed and composite size-distributions.

An attempt was made to estimate the collection efficiency of the two methods used for collection of droplets for counting, namely the catching of droplets on microscope slides or culture plates exposed directly to spray in front of the mouth and the collecting of droplets as nuclei on to microscope slides or culture plates by use of the slit sampler. On the assumption that no droplets of any size were missed by both methods of collection, the collection efficiency of each method was calculated by comparison of the size-distribution series observed by that method with the calculated composite size-distribution series. As it was not certain that some droplets were not missed by both methods of collection, the collection efficiencies calculated in this way are to be regarded as the maximum collection efficiencies of the methods.

The calculation of collection efficiency is shown in Table 16. The calculation was made separately for speech droplets, for cough droplets and for sneeze droplets. Firstly, in Table 16 is shown the calculated composite droplet size-distribution as in Table 15, giving the percentage of all droplets which belongs to each size group. Beside this is shown in Table 16, (a) the size-distribution series for droplets collected as nuclei by the slit sampler (from upper

half/

half of Table 14), and (b) the size-distribution series for droplets caught on slides exposed in front of the mouth (from lower half of Table 14); these series were expressed in terms of percentages of the total number of droplets in the composite series and not, as in Table 14, in terms of percentages of the droplets in the observed series given by the single collection method in question. Thus, series (a) in Table 16 consists of the percentage figures of the observed series for droplets collected as nuclei by the slit sampler (Table 14, upper half) divided by a factor such as to equate those for size groups under 40 microns to the corresponding figures in the composite series (Table 15). Similarly, series (b) in Table 16 consists of the percentage figures of the observed series for droplets caught on slides exposed in front of the mouth (Table 14, lower half) divided by a factor such as to equate those for over 40 microns to the corresponding figures in the composite series (Table 15).

Collection efficiency was given by addition of the percentage figures for all the size groups in the appropriate series (a or b) in Table 16; this gave the percentage of droplets of all sizes in the composite series which was collected by the method in question. It was estimated in this way that 75% of speech droplets, 84% of cough droplets and 97% of sneeze droplets were collected as nuclei by use of the slit sampler/

sampler, and that 41% of speech droplets, 28% of cough droplets and 8% of sneeze droplets were collected on microscope slides or culture plates held in front of the mouth.

When the droplets or nuclei were collected on to microscope slides and were counted microscopically, probably all which were collected were also counted; thus, the efficiencies of the droplet counting methods involving microscopical observation would be the same as the collection efficiencies just quoted.

When the droplets or nuclei were collected on to culture plates and counted as colonies, a proportion would be missed which did not contain viable bacteria and could not give rise to colonies. Table 18 shows the proportion of droplets in each size group which will form colonies if the atomised secretion contains 30,000,000 "viable bacterial units" per milliliter. The saliva of the test subject was infected with commensal bacteria to this extent. The percentages in Table 18 were extracted from the percentages in the corresponding size groups in Table 16 (series a and b) and the products for all size groups were added to give the percentage of all droplets in the composite series which were collected and counted by the cultural method in question. It was estimated in this way that 9% of speech droplets, 10% of cough droplets and 5% of sneeze droplets were collected and counted by the method of collecting/

collecting nuclei with the slit sampler on to culture plates and counting the resulting colonies. It was estimated that 31% of speech droplets, 20% of cough droplets and 5% of sneeze droplets were collected and counted by the method of catching droplets on culture plates exposed in front of the mouth and counting the resulting colonies.

These calculated maximum collection and counting efficiencies of the different methods are summarised in Table 7.

Section 31: Observation of the duration of air carriage and the sedimentation rate of droplet nuclei by repeated slit sampler examinations of the air of a chamber contaminated with droplet spray.

In many of the experiments in which droplet nuclei were counted by sampling the air of a chamber at ½ to 1½ minutes after spray production by coughing or sneezing (Sections 17 and 18), sampling was continued at subsequent intervals so that the "die-away" of air contamination might be observed. In some of the tests a desk-type electric fan was run at half speed during the entire period until the last sample had been taken; in other tests the fan was run only during the first minute after spray production. Tables 21, 23 and 25 give the counts made by the cultural method and show the "die-away" of bacteria-carrying nuclei. Tables 26-43 give the counts made by the microscopical method and show the "die-away" of all microscopically visible nuclei. In these latter experiments the nuclei were measured and size-distribution counts were made for each sample in order to show the differential settling rates of the differently sized nuclei. To enable easy comparison between different experiments, the counts were also calculated on a common percentage basis; Tables 22, 24, 29, 38 and 43 show the counts expressed as percentages of the counts for ½ to 1½ minutes after spray production. In addition, an average "die-away" series was calculated for each set of experiments made under similar conditions; the average was computed of all the air contamination percentage figures relating to each/

each time interval. Graphs 6 and 7 show these average air contamination "die-away" series.

The disappearance rate of nuclei from the chamber air was also presented in terms of equivalent ventilation rate and in terms of equivalent sedimentation rate; these values were calculated from the percentage reductions in air contamination in each of the first four quarter-hour periods after spray production, and they are shown at the foot of Tables 22, 24, 29 and 38. Equivalent ventilation rate is that rate of ventilation which would bring about disappearance of air contamination at the observed rate if ventilation were the sole cause of the disappearance. The equivalent ventilation rate was calculated from the formula given by Wells and Wells (1938); the number of overturns of chamber air per hour was taken as equal to 138 times the difference in the logarithms of two counts divided by the elapsed time in minutes between the two counts. The calculated equivalent ventilation rate varied mainly between 1 and 10 overturns per hour. Equivalent sedimentation rate is that average rate of settling of the air-borne particles which would result in disappearance of air contamination at the observed rate if sedimentation were the sole cause of the disappearance. On the assumption that the circulation rate of the chamber air was sufficiently great to maintain a uniform distribution of the nuclei within the/

the chamber, the equivalent sedimentation rate was calculated as the height of the chamber times the rate of removal expressed as a percentage of the remainder (Phelps and Buchbinder, 1941). The calculated rates varied in the different experiments mainly between 0.0005 and 0.0015 meters per second. These figures, calculated from the numbers of nuclei of all sizes, refer to the sedimentation rate of a hypothetical "average" nucleus for each experiment. An attempt was made to calculate the particular sedimentation rates of nuclei of each different size. As before, the sedimentation rate was calculated as the height of the chamber times the percentage rate of removal; this latter was taken approximately as 100% divided by the time until nuclei of the size in question were no longer found in the air. In experiments made in the 70 cu.ft. chamber without the fan run throughout, nuclei of about 20 microns diameter disappeared from the air within, on average, about 2 minutes after spray production, nuclei of about 10 microns diameter disappeared in about 15 minutes, and nuclei of about 5 microns in diameter disappeared in about 60 minutes. Calculation from these times of 100% removal gave sedimentation rates of 0.014, 0.0019 and 0.00047 meters per second for nuclei with diameters of 20, 10 and 5 microns respectively. These values, recorded in Table 53, were in close agreement with the values calculated from Stokes' law by Wells (1934).

Disappearance/

Disappearance of bacterial air contamination may be brought about by three factors, sedimentation, ventilation and bacterial death. It is thought that the disappearance of air contamination in the present experiments was almost entirely the result of sedimentation. The test chambers were unventilated except for the small spaces around their close fitting doors. Almost certainly their ventilation rates were considerably less than 1 overturn per hour. Air withdrawn for sampling from the 70 cu.ft. chamber never amounted to more than about 0.1 overturns per hour. The equivalent ventilation rates of the observed disappearances were much greater, mainly between 1 and 10 overturns per hour; the observed disappearances must have resulted from a more rapidly acting cause than ventilation. Removal by ventilation probably was appreciable only in the case of the smallest nuclei, under 2 microns in diameter, which sedimented slowly and disappeared from the chamber air at an equivalent ventilation rate of less than 1 overturn per hour on some occasions. The death rate of salivary commensal bacteria in droplet nuclei is not know with certainty, but it is probably slow in comparison with the rate of sedimentation of droplet nuclei. Buchbinder and Phelps (1941) found that the median survival time of a Strept.viridans strain in broth culture droplet nuclei standing in the dark was about 1 day; such a death rate would give disappearance of air contamination at

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an equivalent ventilation rate of about 0.03 overturns per hour, which is much slower than that observed. Bacteria-carrying nuclei demonstrated by the cultural method disappeared from the air more rapidly than Congo red-containing nuclei demonstrated by the microscopical method; this is regarded as due to the larger average size of the bacteria-containing nuclei (see Table 18), and not as due to rapid bacterial death. In experiments made by microscopical observation of Congo red-containing nuclei, the question of bacterial death did not arise, but another factor may have played a part in bringing about apparent disappearance of air contamination. For some reason not yet understood, the Congo red-containing nuclei often turned black within 2 or 3 hours after spray production; after such times only the smallest nuclei were still airborne and these when black were difficult to distinguish from dust particles. The results yielded by the microscopical method for periods later than 1 hour after spray production, may in some cases have been inaccurate.

In the different experiments without the fan run throughout, the maximum duration of air carriage observed varied from 5 to 30 hours for Congo red-containing nuclei demonstrated microscopically (Table 38), and varied from 1 to 2 hours for Strept.viridans-containing nuclei demonstrated culturally (Tables 21 and 23). It was thought that a more prolonged air carriage/

carriage of bacteria-containing nuclei might be observed if the mouth secretions were artificially inoculated so as to contain a much higher concentration of "viable bacterial units" than was supplied by the natural commensal flora; in this way more of the smallest and most slowly settling nuclei would be infected and made capable of cultural recognition. The anterior mouth of the test subject was inoculated with 1 milliliter of a dense suspension of spores of B.mesentericus. The test subject gave 10 simulated sneezes from standing height into the 100 cu.ft. chamber. Sampling with the slit sampler on to nutrient agar plates was repeated at intervals during one day. After incubation of the plates, the distinctive colonies of B.mesentericus were counted. Air contamination with droplet nuclei containing B.mesentericus was found to persist for 8 hours but not for 15 hours. After complete disappearance of B.mesentericus from the chamber air, a person wearing a sterile mask and dust-proof gown entered the chamber and marched ("marked time"); the air was again sampled. This procedure was repeated on several successive days and on each day the air was found to become reinfected with B.mesentericus-containing nuclei raised from floor or walls by the disturbance of the marching. These experiments with B.mesentericus are recorded in Table 54.

Section 32: Calculation of sedimentation rate of droplets and nuclei from counts made microscopically on slides exposed on the floor at 5 feet below the mouth at various intervals after spray production.

The test subject took Congo red or eosin into his mouth and, while standing erect with his mouth 5 feet above the floor, gave a simulated sneeze horizontally forwards. A number of plain glass microscope slides which previously had been placed side by side on the floor 2 to 3 feet in front of the test subject and which were covered by tinplate lids, were uncovered for various durations and at various intervals after the sneezing. The slides were examined microscopically and the droplet marks and droplet nuclei on a known area were counted and measured micrometrically.

The experiments for observation of the settling of the large droplets were made in the 1700 cu.ft. chamber. The slides were exposed for successive 2-second periods following the sneeze. The observations made in two experiments are shown in Tables 45 and 46; the numbers of droplets of each size and the total number of droplets of all sizes which reached the floor in each successive 2-second period, are shown. The droplet mark diameters are given and the diameters of the spherical parent droplets may be calculated as one third of these. The droplets reaching the floor in the successive 2-second periods were progressively smaller in size. The commonest droplets reaching the floor in 1 to 2 seconds had a diameter (spherical) of about/

about 200 microns at the moment of impact. According to Wells (1934) a water droplet of 200 microns diameter takes 7 seconds to evaporate completely in unsaturated air at 18°C. Thus, a saliva droplet of 200 microns diameter would shrink very little during the 1 to 2 seconds while it fell from mouth to floor, so that its diameter as calculated at the moment of reaching the floor may be taken to represent approximately its diameter at the moment of leaving the mouth. From the observation that 200-micron droplets took 1 to 2 seconds for falling through 5 feet, the sedimentation rate of 200-micron droplets was calculated to be between 0.85 and 1.7 meters per second; this value, recorded in Table 53, agrees with the value calculated from Stokes' law (see Wells, 1934). The commonest droplets reaching the floor in 2 to 4 seconds had a calculated spherical diameter of about 100 microns at the moment of impaction. According to Wells (1934), a 100-micron water droplet in unsaturated air at 18°C. requires only 1.7 seconds for complete evaporation. Thus, although saliva droplets probably evaporate more slowly than water droplets, it is not unlikely that the droplets which were calculated to be 100 microns in diameter at the moment of reaching the floor were appreciably larger at the moment of leaving the mouth. It is not surprising, therefore, that the sedimentation rate calculated for these "100-micron" droplets, namely 0.43-0.85 meters per second, was higher than that calculated from Stokes' law, 0.3 meters per second

(see/

(see Table 53). Between 4 and 14 seconds after the sneeze, even smaller droplets reached the floor. These were calculated as ranging from 25 to 100 microns in diameter at the moment of reaching the floor. They must have been much larger at the time of leaving the mouth, perhaps from 100 to 150 microns in diameter.

The experiments for observation of the settling of droplet nuclei were made in the 100 cu.ft. chamber. The slides were exposed for 1 hour or 18 hours. Table 47 shows the numbers of nuclei of each size found on the slides in four experiments. It was assumed that nuclei of the smallest size found on the slide must have settled from the air on to the floor in large numbers. The time of 50% removal by sedimentation of nuclei of this size was taken to be very approximately the duration of exposure to sedimentation. Thus the sedimentation rate of nuclei of this size was calculated as being of the order of 1.7 meters divided by the duration of exposure to sedimentation. Nuclei of 5 microns diameter were the smallest found after sedimentation for 1 hour; their sedimentation rate was calculated as about 0.00047 meters per second. Nuclei of 1 micron diameter were the smallest found after sedimentation for 18 hours; their sedimentation rate was calculated as about 0.000025 meters per second. This method of calculation is of doubtful validity, and the close agreement between these calculated rates and the values derived from Stokes' law (see Table 53) may be fortuitous.

Section 33: Observation of time required for droplet evaporation and droplet nucleus formation.

Method (i): Microscopical observation of drops hanging from glass fibres. Special experiments were described in Section 25 for determining the amount of shrinkage occurring in droplet nucleus formation. Drops of tap water, drops of saliva and drops of saliva with 1-2% Congo red were suspended from the ends of glass fibres held in the low power field of a horizontally placed microscope. The diameters of these drops were measured by use of the eyepiece micrometer scale. The time of complete evaporation was measured, until the water drops had disappeared entirely and until the residues of the saliva drops had ceased to shrink. Concurrent wet and dry bulb temperature readings were made, and the relative humidity calculated. Table 13 shows the observed times of complete evaporation of the differently sized drops. The times, even for plain water drops, are greater than those given by Wells (1934).

Method (ii): Microscopical observation of evaporation of droplets on slides after impingement from mouth spray. A glass or celluloid slide was placed on the stage of the microscope. The observer applied his eye to the microscope and focussed the surface of the slide with the low power (2/3in.) objective and micrometer eyepiece. The test subject stood in front of the microscope with his mouth about 1 foot above the slide, and/

and from this position gave a simulated sneeze towards the slide. The observer started a stop watch at the moment of the sneeze. If a droplet landed on the part of the slide seen through the microscope, the observer watched and timed the evaporation of that droplet; evaporation was regarded as complete when the fluid edge had completed its retraction centrally and crystalline deposit was apparent at the centre. The diameter of the droplet deposit mark was measured micrometrically and the diameter of the spherical parent droplet was calculated. Table 52 shows the evaporation times thus observed for differently sized droplets. It is to be noted that the conditions of evaporation of a droplet flattened on a slide may be very different from those of a droplet flying through air. However, except for the largest droplets, these times agreed closely with the times observed and and calculated by other methods.

Method (iii): Examination of chamber air for dye-containing nuclei by momentary sampling with the slit sampler at short intervals after spray production. The test subject took 0.1 grams of Congo red into his mouth and gave a simulated sneeze into the 2½ cu.ft. chamber or the 1700 cu.ft. chamber. The fan was not used. A stop watch was started at the moment of sneezing. After a measured interval of 0, 1, 2, 3 or 4 seconds, the slit sampler was run during the course of one second to take about 1/60 cu.ft. of air on to an unoiled/

unoiled slide. The slide was examined microscopically and the droplet marks and droplet nuclei were counted and measured micrometrically. The droplet nuclei were readily distinguished from the marks of droplets which were still liquid at the moment of sampling; the latter were lighter in colour, without depth of focus and much greater in diameter (see Section 14 and Frontispiece). This distinction was maximised by the use of unoiled slides which allowed free spreading of the liquid droplets. The unoiled surface probably did not retain as large a proportion of the nuclei as did an oiled surface. In some experiments, a second "slit sampler" was used so that two samples might be taken in close succession. Tables 48-51 record the observations made. The largest nucleus observed in the first second after sneezing was about 11 microns in diameter (i.e. from a parent droplet of about 44 microns diameter), and the largest nucleus observed in the second second after sneezing was about 15 microns in diameter (i.e. from a parent droplet of about 60 microns diameter). This suggested that in natural droplet spray a 44-microns droplet evaporates to a nucleus within 1 second and a 60-microns droplet evaporates to a nucleus within 2 seconds; the time of evaporation of a 50-microns droplet is indicated as being between 1 and 2 seconds.

When a longer time was available for evaporation, larger/

larger droplets became airborne nuclei. In the mouth spray experiments described in Sections 23 and 24, when the falling height was 5 feet and unlimited time (over a ½ minute) was available for evaporation, the largest nuclei found in the air with rare exceptions were less than 25 microns in diameter (Tables 11 and 47); the largest droplet capable of becoming a nucleus while still airborne was thus about 100 microns in diameter (25 x 4). Between 10 and 20 seconds was the longest time available for evaporation and nucleus formation by the droplets before they fell on the floor; in the experiments described in Section 32, the smallest and most slowly falling droplets which reached the floor still not completely evaporated, were observed to reach the floor at about 15 seconds after spray production (Tables 45 and 46). Slightly smaller droplets evaporating in a slightly shorter time, say 10 seconds, would be the largest droplets capable of becoming nuclei while still airborne; for the reasons given above, these were thought to be about 100 microns in diameter. Thus, the evaporation time of a 100-micron droplet was about 10 seconds.

Comparison of Evaporation Times Observed and Calculated by Different Methods. To facilitate comparison, the droplet evaporation times which were observed and calculated by the different methods, were assembled in a single table, Table 55. This table shows for each droplet size and each method of observation the average observed/

observed time for complete evaporation (Columns 2-6). The overall average for each droplet size of the values given by all methods (Columns 2-6), corrected so as to be proportional to the square of the droplet diameter, are shown in Column 7. These observed evaporation times for mouth spray droplets at about 16°C. and 60% relative humidity are 7 to 8 times larger than the times calculated by Wells (1934) for water droplets at 18°C. in unsaturated air.

Section 34: TABLES, GRAPHS AND FIGURES.

Tables Presenting Original Observations:-

1, 2, 3, 4, 5, 6, 8, 9, 11, 13, 20, 21, 23, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41, 42, 45, 46, 47, 48, 49, 50, 51, 52 and 54.

Tables Presenting Values Derived by Calculation or Composition from Original Observations:-

7, 10, 12, 14, 15, 16, 17, 18, 19, 22, 24, 29, 38, 43, 44, 53 and 55.

Table 1:- Number of Colonies Obtained on a 12-sq.in. Blood Agar Plate Exposed 3 in. in front of Mouth and Nose.
(Each figure given is the result of a different test. Fifteen tests were made with each activity.)

Test Subject	Mouth Breathing for 1 Minute*	Laughing for 1 Minute*	Speaking Loudly 100 "K.s" *	Speaking Softly 100 Words*	Speaking Loudly 100 Words*	Single 'Throat-only Cough'			Single 'Lip Cough'	Single 'Tongue-teeth Cough'	Nose Breathing for 1 Minute†	Single Strong Nasal Expiration	Single Natural Sneeze, Mouth Masked.
A	0	6	650	30	284	1100	225	56	1300	6500	'0'	1200	185
	0	4	198	13	282	55	29	29	1200	2480	'0'	1000	24
	0	3	153	13	198	27	26	25	880	2200	'0'	600	18
	0	2	25	12	169	22	21	21	588	1400	'0'	450	12
	0	1	23	6	120	21	19	19	470	1340	'0'	398	11
	0	0	18	6	117	15	12	11	322	1200	'0'	275	10
	0	0	15	4	116	11	11	3	281	800	'0'	56	6
	0	0	2	4	115	0	0	0	205	640	'0'	33	5
	0	0	1	3	81	0	0	0	168	610	'0'	17	4
	0	0	0	2	70	0	0	0	110	400	'0'	9	3
B	0	3	2	1	21	23	10	8	1344	720	'0'	0	-
C	0	0	0	3	1	2	0	0	336	235	'0'	17	-
D	0	0	56	8	15	157	145	14	15	1570	'0'	19	-
E	0	0	0	0	9	7	6	0	58	21	'0'	24	-
F	0	0	0	21	74	17	2	0	122	475	'0'	4	-
Average:	0	1	76	8	110		48		490	1380	'0'	280	28
	Mainly Mouth Spray										Nose Spray Only		

*number of Strept.viridans colonies only. †counts not significantly greater than control counts (about 10).

Table 2:- Number of Droplets Caught on Slides Exposed 6 Inches in front of the Mouth.

(Given as the counts of droplet marks found on a 1-sq.in. slide multiplied by 20 for sneezes, and by 10 for coughs and speaking. The mouth was treated with Congo Red and the droplet marks were recognised by their colour; the counts thus relate to Mouth Spray only. All tests were carried out with subject A.)

Expiratory Activity	Speaking Loudly 100 Words	Single 'Throat-only Cough'	Single 'Tongue-teeth Cough'	Single 'Lip Cough'	Single Natural Sneeze
Results of twelve tests with each Activity	550	1100	7100	5800	52,000
	490	180	5900	5200	46,000
	420	40	2200	2500	44,000
	410	40	1500	2500	39,000
	340	10	1100	1800	33,000
	340	10	900	1500	26,000
	180	0	830	1400	25,000
	140	0	480	1100	17,000
	90	0	440	1000	11,000
	90	0	350	820	8,000
	50	0	340	490	5,000
	40	0	30	360	3,700
Average:	260	120	1800	2000	26,000

Table 3:- Number of Bacteria-carrying Droplet Nuclei in Air of Chamber at ½ to 1½ Minutes after Expiratory Act.

(Computed from colony counts on blood agar plates exposed in the slit sampler. Each figure is the result of a different test. All tests were carried out with subject A.)

Speaking Loudly 100 "K's"	Speaking Softly 100 Words	Speaking Loudly 100 Words	Single 'Throat-only Cough' †	Single 'Tongue-teeth Cough'	Single 'Lip Cough'	Single Natural Sneeze	Single Violent Simulated Sneeze
30*	35*	210	6*	1500	3500	150,000	1,000,000
20*	25*	190	5*	1200*	2000	110,000	800,000
10*	25*	150	5*	1000*	1800	90,000	700,000
5*	20*	130	3*	900*	1200*	80,000	700,000
0*	20*	100*	0*	900*	1000*	35,000*	500,000
0*	20*	90*				30,000*	400,000
0*	15*	85*				30,000*	350,000
0*	15*	85*	80	1300	850	30,000*	250,000
7 Av.	15*	65*	32	510	560	20,000*	250,000
	15*	60*	11*	470	530	10,000*	250,000
	15*	55*	11*	410	470		200,000
Strong Nasal Expiration.†	15*	55*	5*	310	190	50,000	200,000
65	10*	50*	4*			42,000	170,000
45	10*	45*	2*			31,000	150,000
18	10*	45*	2*	1500	490	30,000	150,000
10	5*	40*	2*	1300	450	15,000	150,000
5	5*	35*	2*	1100	310	15,000	150,000
0	0*	35*	1*	350	110*	11,000	140,000
0	0*	35*	1*	340	70*	9,000	140,000
0	0*	15*	1*	340	45*	8,500	130,000
0	0*	10*	0*	180*	20*	6,500	130,000
0	0*	5*	0*	150*	10*	4,500	120,000
16 Av.	0*		0*	80*	5*		120,000
Average:-	13	71	8	730	720	39,000	310,000

In 2½ cu.ft. — In 2½ cu.ft. Chamber — 70 cu.ft. — 1700 cu.ft † — 70 cu.ft † — 2½ cu.ft. — 1700 cu.ft † — In 1700 cu.ft. — In 70 cu.ft. — 2½ cu.ft. — In 1700 cu.ft. Chamber

*computed from counts of Strept.viridans colonies only. †computed from average of 5-50 coughs in each test

Table 4:- Number of Dye-containing Droplet Nuclei in Air of Chamber at ½ to 1½ Minutes after Expiratory Act.

(Computed from counts of colored droplet nuclei observed with the microscope on oiled slides exposed in the slit sampler. Each figure is the result of a different test. All tests were made with subject A.)

Speaking Softly 100 Words	Speaking Loudly 100 Words	Single 'Tongue-teeth Cough'	Single 'Lip Cough'	Single Natural Sneeze	Single Violent Simulated Sneeze.
160	770	6500	6000	1,500,000	16,000,000
120	490	5500	6000	1,300,000	15,000,000
110	410	4500	4000	1,100,000	14,000,000
100	400	4000	3000	1,100,000	12,000,000
95	310			950,000	10,000,000
95	280			200,000	8,500,000
90	270	2100	1600		8,500,000
85	260	1500	810		8,000,000
70	260			3,100,000	6,500,000
60	250			2,600,000	4,500,000
50	220	52,000	16,000	1,900,000	4,000,000
40	210	13,000	16,000	1,800,000	4,000,000
35	190	9800	8600	1,200,000	
35	170	8900	4000	1,000,000	
30	160	7900	3500	880,000	30,000,000
25	160	4200	2400	640,000	9,000,000
25	95	3800	1900	580,000	8,500,000
25	85	3700	1800	470,000	4,500,000
15	75	2000	970	220,000	3,500,000
0	50	2000	490	65,000	1,500,000
Average:- 63	250	8200	4800	1,100,000	9,300,000

In 2½ cu.ft. Chamber | In 2½ cu.ft. Chamber | In 1700 cu.ft † | In 1700 cu.ft † | In 70 cu.ft. | In 70 cu.ft. | In 1700 cu.ft. | In 2½ cu.ft.

† - computed from average for 5-50 coughs in each test.

Table 5:- Number of B.prodigiosus Colonies on a 12-sq.in. Agar Plate Exposed 3 Inches in front of the Mouth after Artificial Inoculation of B.prodigiosus into either the Throat or the Nose.

(Each figure is the result of a different test; 15 or 30 tests were made with each activity.)

Test Subject	Laughing for 1 Minute	Speaking Loudly 100 "K's"	Single 'Throat-only Cough'		Single Natural Sneeze	Nose Breathing for 5 Minutes		Single Natural Sneeze	
A	6	220	160	130	2300	6	6	5600	710
	3	30	66	28	510	6	3	332	209
	3	22	18	15	390	3	2	106	36
	2	2	11	8	65	2	2	33	23
	2	2	8	7	37	2	2	16	6
	2	1	6	4	27	1	1	2	2
	1	1	4	2	11	1	0	2	2
	0	0	2	2	10	0	0	2	1
	0	0	1	1	8	0	0	1	1
	0	0	0	0	0	0	0	0	0
B	0	0	23	3	22	5	4	4	0
C	0	0	108	6	920	1	1	51	6
D	0	2	279	22	827	0	0	6	5
E	0	9	2	0	97	3	0	72	3
F	12	1100	116	8	70	1	0	212	0
Average:-	2	92	31		360	2		250	
	Droplets of Throat-origin Only					Droplets of Nose-origin Only			
	B.prodigiosus applied only to tonsillar areas, edge of soft palate and back of tongue; anterior mouth proved free of B.prodigiosus.					B.prodigiosus applied only to the anterior nasal passages.			

Table 6:- Number of Droplet Nuclei Containing B.prodigiosus in the Air of the 2½-Cu.Ft. Chamber at ½ to 1½ Minutes after the Expiratory Act.

(Computed from B.prodigiosus colony counts on nutrient agar plates exposed in the slit sampler. Each figure is the result of a different test. All tests were carried out with subject A.)

	Speaking Loudly 100 "K's"	Single 'Throat-only Cough' *	Single Natural Sneeze	Nose Breathing for 5 Minutes †	Single Natural Sneeze
	33	5.3	390	5	360
	15	2.6	270	4	75
	8	2.5	220	2	55
	5	2.2	140	1	25
	5	1.6	110	1	10
	3	0.8	8	1	10
	3	0.8	0	1	10
	1	0.6	0	0	10
	0	0.2	0	0	5
	0	0.2	0	0	5
Average:-	7	2	110	2	56
	Droplet Nuclei of Throat-origin Only			Droplet Nuclei of Nose-origin Only	
	B.prodigiosus applied only to tonsillar areas, edge of soft palate and back of tongue; proved absent from front of mouth			B.prodigiosus applied only to anterior nares and anterior nasal cavity.	

* computed as average for one cough from count for volley of 30 coughs in each test.

† air sampling was done during the time of the breathing.

Table 7:- Average Numbers of Droplets Expelled by Expiratory Activities as Counted by the Four Different Methods

Counting Method:-	(i) Counting Colonies on 12-Sq.In. Culture Plates Exposed 3 Inches in front of the Mouth	(ii) Counting Droplet Marks on Slides Exposed 6 Inches in front of the Mouth	(iii) Counting Colonies on Culture Plates Exposed in Slit Sampler to Air of Chamber Contaminated by Droplet Spray ½-1½ min.	(iv) Counting Microscopically the Colored Droplet Nuclei on Oiled Slides Exposed in Slit Sampler to Air of Chamber Contam. by Spray
Type of Droplets Counted:-	Bacteria-Carrying Droplets Larger than about 20 microns in diameter (5% - 30% of total number)	All Droplets Larger than about 20 microns in diam. (8% - 40% of total number)	Bacteria-Carrying Droplets Small Enough to Form Droplet Nuclei, i.e. orig. under 100 microns (5% - 10% of total)	All Droplets Small Enough to Form Droplet Nuclei, i.e. originally under 100 microns in diameter (75%-97% of total)
Mouth Breathing for 1 Min.	0	-	-	-
Laughing for 1 Minute.	1 (T= 2)	-	-	-
Nose Breathing for 5 Min.	'0' (N= 2)	-	- (N= 2)	-
1 Strong Nasal Expiration.	280	-	16	-
Speaking Loudly 100 "K's".	76 (T=92)	-	7 (T= 7)	-
Speaking Softly 100 Words.	8	-	13	63
Speaking Loudly 100 Words.	110	260	71	250
Single 'Throat-only Cough'	48 (T=31)	120	8 (T= 2)	-
Single 'Tongue-teeth Cough'	1400	1800	730	8200
Single 'Lip Cough'.	490	2000	720	4800
Single Natural Sneeze.	-(T=360, N=250)	26,000	39,000 (T=110, N=56)	1,100,000
Single Simulated Sneeze.	-	-	310,000	9,300,000

Numbers shown are the average counts taken from tables 1-6. The numbers in brackets are the average counts obtained in the tests in which only B.prodigiosus colonies were counted: T = throat droplets, N = nose droplets

Table 8:- Diameters in Millimeters of Hanging, Nearly Spherical Saliva Droplets and Diameters of their Marks when Fallen and Dried on a Slide.

4 Droplets Falling on Glass Slides			10 Droplets Falling on Celluloid Slides		
Hanging Droplet	Droplet Mark	Ratio	Hanging Droplet	Droplet Mark	Ratio
2.0	6.0	1:3	2.0	5.0	1:2½
1.5	4.5	1:3	2.0	5.0	1:2½
1.5	4.5	1:3	2.0	5.0	1:2½
1.0	3.0	1:3	1.6	3.2	1:2
			1.5	3.0	1:2
			1.3	2.5	1:2
			1.3	2.5	1:2
			1.2	2.2	1:2
			1.2	2.0	1:1¾
			1.0	2.0	1:2
	Average Ratio:-	1:3		Average Ratio:-	1:2

Table 9:- Number of Droplets of Each Size Caught on Celluloid Slides Exposed 6 Inches in front of the Mouth.

(For each type of expiratory activity the diameters of 3000 droplet marks were measured. The diameters of the original spherical droplets were calculated as being half the diameters of the droplet marks.)

Droplet Mark Diameters in Microns	Speaking Loudly 100 Words	'Throat-only Cough'	'Lip Cough' or 'Tongue-teeth Cough'	Natural Sneeze	Simulated Sneeze	Calculated Diameters of Spherical Parent Droplets in Microns
0 - 10	0	0	0	0	0	0 - 5
10 - 20	20	8	24	36	37	5 - 10
20 - 30	84	39	119	94	122	10 - 15
30 - 40	200	127	337	267	345	15 - 20
40 - 50	224	189	346	312	352	20 - 25
50 - 100	597	577	767	807	1088	25 - 50
100 - 150	531	593	468	593	509	50 - 75
150 - 200	352	341	285	260	236	75 - 100
200 - 250	260	231	160	144	109	100 - 125
250 - 300	214	202	125	105	56	125 - 150
300 - 400	179	253	115	115	72	150 - 200
400 - 500	99	165	96	82	51	200 - 250
500 -1000	197	213	113	118	22	250 - 500
1000 -2000	41	52	40	59	1	500 -1000
2000 -4000	2	10	5	8	0	1000 -2000
Number of Experiments:-	21	22	14	10	7	

Table 10:- Percentage of Droplets of Each Size Caught on Celluloid Slides Exposed 6 Inches in front of Mouth.

(Percentages calculated from size-distribution counts recorded in table 9.)

Droplet Mark Diameter in Microns	Speaking Loudly 100 Words	'Throat-only Cough'	'Lip Cough' or 'Tongue-teeth Cough'	Natural Sneeze	Simulated Sneeze	Calculated Diameter of Spherical Parent Droplet in Microns
0 - 10	0	0	0	0	0	0 - 5
10 - 20	0.67	0.27	0.8	1.2	1.2	5 - 10
20 - 30	2.8	1.3	4.0	3.1	4.1	10 - 15
30 - 40	6.7	4.2	11.2	8.9	11.5	15 - 20
40 - 50	7.5	6.3	11.5	10.4	11.7	20 - 25
50 - 100	19.9	19.2	25.6	26.9	36.3	25 - 50
100 - 150	17.7	19.8	15.6	19.8	17.0	50 - 75
150 - 200	11.7	11.4	9.5	8.7	7.9	75 - 100
200 - 250	8.7	7.7	5.3	4.8	3.6	100 - 125
250 - 300	7.1	6.7	4.2	3.5	1.9	125 - 150
300 - 400	6.0	8.4	3.8	3.8	2.4	150 - 200
400 - 500	3.3	5.5	3.2	3.7	1.7	200 - 250
500 -1000	6.6	7.1	3.8	3.9	0.73	250 - 500
1000 -2000	1.4	1.7	1.3	2.0	0.03	500 -1000
2000 -4000	0.07	0.33	0.17	0.27	0	1000 -2000
All Sizes:-	100%	100%	100%	100%	100%	All Sizes

Table 11:- Number of Congo Red-Containing Droplet Nuclei of Each Size Measured Micrometrically on Oiled Slides Exposed in the Slit Sampler to Air of Chamber at ½ to 1½ Minutes after Expiratory Act.

Expiratory Act:-	Speaking Softly 100 Words	Speaking Loudly 100 Words	'Throat-only Cough'	'Tongue-teeth Cough'		'Lip Cough'		Natural Sneeze		Simulated Sneeze	
Chamber Capacity:-	2½ c.f..	2½ c.f.	2½ c.f.	1700 c.f & 70 c.f	2½ c.f.	1700 c.f & 70 c.f	2½ c.f	1700 c.f & 70 c.f	2½ c.f	1700 c.f & 70 c.f	2½ c.f.
Falling Ht.-	1½ ft.	1½ ft.	1½ ft.	5 ft.	1½ ft.	5 ft.	1½ ft.	5 ft.	1½ ft.	5 ft.	1½ ft.
No.of Tests-	18	22	4 ¢	10	9	5	10	17	3	6	9
Nucleus Diameter in Microns: ¼ - ½	0	10	0	0	31	8	37	49	215	24	326
½ - 1	3	115	43	17	176	38	204	729	904	503	474
1 - 2	28	455	520	100	604	114	634	1586	1931	670	669
2 - 4	66	677	849	395	841	331	872	1627	1270	468	375
4 - 6	35	351	362	195	444	191	477	574	420	177	110
6 - 8	26	213	143	112	232	119	172	227	153	76	33
8 - 10	10	110	55	77	131	78	73	112	64	40	9
10 - 12	4	49	20	41	33	37	21	52	25	19	1
12 - 14	3	16	7	27	7	27	8	23	10	10	1
14 - 16	0	3	1	20	1	17	1	12	5	9	1
16 - 18	0	1	0	10	0	12	0	6	3	3	1
18 - 20	0	0	0	4	0	15	1	3	0	1	0
20 - 25	0	0	0	1	0	10	0	0	0	0	0
25 - 50	0	0	0	1#	0	3#	0	0	0	0	0
All Sizes:-	175	2000	2000	1000	2500	1000	2500	5000	5000	2000	2000

28, 32, 35 and 42 microns.

¢ 120 coughs in the 4 tests.

Table 12:- Percentage of Droplet Nuclei of Each Size Found in Air at ½ to 1½ Minutes after Expiratory Act.
(Percentages calculated from size-distribution counts recorded in table 11.)

Expiratory Act:-	Speaking Softly 100 Words	Speaking Loudly 100 Words	'Throat-only Cough'	'Tongue-teeth Cough'		'Lip Cough'		Natural Sneeze		Simulated Sneeze	
Chamber Capacity:-	2½ c.f.	2½ c.f.	2½ c.f.	1700 c.f & 70 c.f	2½ c.f.	1700 c.f & 70 c.f	2½ c.f	1700 c.f & 70 c.f	2½ c.f	1700 c.f & 70 c.f	2½ c.f.
Falling Ht.-	1½ ft.	1½ ft.	1½ ft.	5 ft.	1½ ft.	5 ft.	1½ ft.	5 ft.	1½ ft.	5 ft.	1½ ft.
No.of Tests-	18	22	4	10	9	5	10	17	3	6	9
Nucleus Diameter in Microns: ¼ - ½	0	0.5	0	0	1.2	0.8	1.5	0.98	4.3	1.2	16.3
½ - 1	1.7	5.8	2.2	1.7	7.1	3.8	8.2	14.6	18.1	25.2	23.7
1 - 2	16.0	22.8	26.0	10.0	24.2	11.4	25.4	31.7	38.6	33.5	33.5
2 - 4	37.8	33.9	42.5	39.5	33.7	33.1	35.0	32.5	25.4	23.4	18.8
4 - 6	20.0	17.6	18.1	19.5	17.8	19.1	19.1	11.5	8.4	8.9	5.5
6 - 8	14.9	10.7	7.2	11.2	9.3	11.9	6.9	4.5	3.1	3.8	1.7
8 - 10	5.7	5.5	2.8	7.7	5.3	7.8	2.9	2.2	1.3	2.0	0.45
10 - 12	2.3	2.5	1.0	4.1	1.3	3.7	0.84	1.0	0.5	0.95	0.05
12 - 14	1.7	0.80	0.35	2.7	0.28	2.7	0.32	0.46	0.20	0.50	0.05
14 - 16	0	0.15	0.05	2.0	0.04	1.7	0.04	0.24	0.10	0.45	0.05
16 - 18	0	0.05	0	1.0	0	1.2	0	0.12	0.06	0.15	0.05
18 - 20	0	0	0	0.4	0	1.5	0.04	0.06	0	0.05	0
20 - 25	0	0	0	0.10	0	1.0	0	0	0	0	0
25 - 50	0	0	0	0.10	0	0.3	0	0	0	0	0
All Sizes:-	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table 13:- Time of Evaporation and Diameters Before and After Evaporation of Droplets Hanging from Glass Fibres Observed Microscopically

	Initial Diameter in Microns	Final Diameter in Microns	Ratio of Diameters	Time of Evaporation in Minutes	Temp. Far.	Rel. Hum.
12 Drops of Saliva & Congo Red	1000	340	2.9 to 1	30½	63°	58%
	1140	330	3.5 to 1	28½	63°	58%
	980	300	3.3 to 1	26	63°	58%
	1224	400	3.1 to 1	37	63°	60%
	1230	320	3.8 to 1	36½	63°	60%
	1310	280	4.7 to 1	37	63°	60%
	1110	305	3.6 to 1	32	59°	57%
	1360	270	5.0 to 1	37½	61°	58%
	1300	220	5.9 to 1	-	59°	61%
	1450	350	4.1 to 1	60	59°	61%
	530	150	3.5 to 1	14	58°	75%
	1050	340	3.1 to 1	44	58°	75%
		Average:-	3.9 to 1			
11 Drops of Saliva	950	290	3.3 to 1	25	63°	58%
	1350	230	5.9 to 1	37	63°	60%
	1224	300	4.1 to 1	29	59°	57%
	1090	250	4.4 to 1	25	61°	58%
	520	70	7.4 to 1	6½	61°	58%
	1275	306	4.2 to 1	35	61°	58%
	1360	250	5.4 to 1	-	59°	61%
	1275	180	7.1 to 1	30	59°	61%
	440	120	3.7 to 1	4½	59°	61%
	1200	210	5.7 to 1	26	59°	61%
	990	160	6.2 to 1	36	58°	75%
		Average:-	5.2 to 1			
12 Drops of Water	868	0		18	63°	58%
	990	0		21½	63°	58%
	1000	0		25	59°	57%
	1090	0		23	61°	58%
	500	0		6½	61°	58%
	1000	0		29	61°	58%
	500	0		5½	61°	58%
	1360	0		37	61°	58%
	1275	0		34	61°	58%
	1620	0		56	61°	58%
	1000	0		24	61°	58%
	500	0		7	61°	58%

Table 14:- Percentages of Droplets and Nuclei of Each Size: Composited from Tables 9 and 11.

	Measured Diameter in Microns	SPEAKING (loudly) %	COUGHING (all kinds) %	SNEEZING (natural & simulated) %	Calculated Diameter of Parent Droplet in Microns
Table 11: Droplet Nuclei		(of 2000)	(of 9000)	(of 14000)	
	¼ - ½	0.50	0.85	4.4	1 - 2
	½ - 1	5.8	5.3	18.6	2 - 4
	1 - 2	22.8	21.9	34.8	4 - 8
	2 - 4	33.9	36.6	26.7	8 - 16
	4 - 6	17.6	18.5	9.1	16 - 24
	6 - 8	10.7	8.6	3.5	24 - 32
	8 -10	5.5	4.6	1.6	32 - 40
	10-12	2.5	1.7	0.69	40 - 48
	12-14	0.80	0.85	0.31	48 - 56
	14-16	0.15	0.45	0.19	56 - 64
	16-18	0.05	0.25	0.09	64 - 72
	18-20	0	0.23	0.03	72 - 80
	20-25	0	0.12	0	80 -100
	25-50	0	0.05	0	100 -200
Table 9: Droplet Marks		(of 3000)	(of 6000)	(of 6000)	
	0 -10	0	0	0	0 - 5
	10-20	0.67	0.53	1.2	5 - 10
	20-30	2.8	2.6	3.6	10 - 15
	30-40	6.7	7.7	10.2	15 - 20
	40-50	7.5	8.9	11.2	20 - 25
	50-100	19.9	22.5	31.7	25 - 50
	100-150	17.7	17.8	18.5	50 - 75
	150-200	11.7	10.5	7.9	75 -100
	200-250	8.7	6.5	4.2	100 -125
	250-300	7.1	5.4	2.7	125 -150
	300-400	6.0	6.1	3.1	150 -200
	400-500	3.3	4.3	2.2	200 -250
	500-1000	6.6	5.4	2.3	250 -500
	1000-2000	1.4	1.5	1.0	500 -1000
	2000-4000	0.07	0.25	0.13	1000-2000

Table 15:- Calculated Composite Size Distribution of Droplets Expelled in Speaking, Coughing and Sneezing: Percentage in Each Size Group.

(For parent droplet diameters under 40 microns, the size distribution calculated from droplet nucleus measurements was taken: i.e. as in tables 11 and 14. For parent droplet diameters over 40 microns, the size distribution calculated from droplet mark measurements was taken: i.e. as in tables 9 and 14. The two size distributions were equated at the 40-micron abscissa by applying the ratios given by the percentage distribution curves, graphs 3, 4, 5)

Method	Calculated Diameter of Parent Droplet in Microns.	SPEAKING (loudly)	COUGHING (all kinds)	SNEEZING (natural and simulated)
Calculated from Nucleus Measurements: tables 11,	1 - 2	0.37%	0.72%	4.2%
	2 - 4	4.3%	4.4%	18.0%
	4 - 8	17.0%	18.0%	34.0%
	8 - 16	25.0%	31.0%	26.0%
	16 - 24	13.0%	16.0%	8.8%
	24 - 32	7.9%	7.3%	3.4%
	32 - 40	4.0%	3.9%	1.5%
Calculated from Droplet Mark Measurements: tables 9 and	40 - 50	3.2%	2.4%	0.87%
	50 - 75	7.2%	5.0%	1.5%
	75 - 100	4.8%	2.9%	0.64%
	100 - 125	3.5%	1.8%	0.34%
	125 - 150	2.9%	1.5%	0.22%
	150 - 200	2.4%	1.7%	0.25%
	200 - 250	1.3%	1.2%	0.18%
	250 - 500	2.7%	1.5%	0.18%
	500 -1000	0.57%	0.42%	0.08%
	1000-2000	0.03%	0.07%	0.01%
All under 100 Microns		87%	92%	99%

Table 16:- Proportion of Droplets in Each Size Group of the Calculated Composite Distribution which Were Collected by the Two Different Methods: (a) by Recovery of Droplet Nuclei from the Air with the Slit Sampler, and (b) by Catching Droplets Directly on Slides Exposed in front of the Mouth.

(Each figure given in the table represents a percentage of the total number of droplets in the composite series. The figures were calculated from those in tables 14, and 15.)

Calculated Diameter of Parent Droplet in Microns	SPEAKING			COUGHING			SNEEZING		
	Composite Size Distribution Percentage	Percentage by (a): 'Nuclei from Air'	Percentage by (b): 'Droplet Marks'	Composite Size Distribution Percentage	Percentage by (a): 'Nuclei from Air'	Percentage by (b): 'Droplet Marks'	Composite Size Distribution Percentage	Percentage by (a): 'Nuclei from Air'	Percentage by (b): 'Droplet Marks'
1 - 2	0.37	0.37	0	0.72	0.72	0	4.2	4.2	0
2 - 4	4.3	4.3	0	4.4	4.4	0	18.0	18.0	0
4 - 8	17.0	17.0	0.1	18.0	18.0	0.07	34.0	34.0	0.06
8 - 16	25.0	25.0	1.4	31.0	31.0	0.9	26.0	26.0	0.41
16 - 24	13.0	13.0	4.0	16.0	16.0	3.1	8.8	8.8	1.3
24 - 32	7.9	7.9	3.9	7.3	7.3	3.0	3.4	3.4	1.2
32 - 40	4.0	4.0	2.9	3.9	3.9	2.2	1.5	1.5	0.89
40 - 50	3.2	2.2	3.2	2.4	1.6	2.4	0.87	0.80	0.87
50 - 75	7.2	0.6	7.2	5.0	1.1	5.0	1.5	0.48	1.5
75 - 100	4.8	0	4.8	2.9	0.3	2.9	0.64	0.03	0.64
100 - 125	3.5	0	3.5	1.8	0.04	1.8	0.34	0	0.34
125 - 150	2.9	0	2.9	1.5	0	1.5	0.22	0	0.22
150 - 200	2.4	0	2.4	1.7	0	1.7	0.25	0	0.25
200 - 250	1.3	0	1.3	1.2	0	1.2	0.18	0	0.18
250 - 500	2.7	0	2.7	1.5	0	1.5	0.18	0	0.18
500 -1000	0.57	0	0.57	0.42	0	0.42	0.08	0	0.08
1000-2000	0.03	0	0.03	0.07	0	0.07	0.01	0	0.01
All Sizes:-	100%	75%	41%	100%	84%	28%	100%	97%	8%

Table 17:- Number of Droplets of Each Size Expelled (a) by Speaking 100 Words, (b) by a Cough and (c) by a Sneeze.

(Calculated from percentages in table 15)

	Calculated Diameter of Parent Droplet in Microns	Speaking Loudly 100 Words	One Cough	One Natural Sneeze
Remain Airborne As Droplet Nuclei	1 - 2	1	7	42,000
	2 - 4	4	44	180,000
	4 - 8	17	180	340,000
	8 - 16	25	310	260,000
	16 - 24	13	160	88,000
	24 - 32	8	73	34,000
	32 - 40	4	39	15,000
	40 - 50	3	24	8,700
	50 - 75	7	50	15,000
	75 - 100	5	29	6,400
Fall At Once To Ground	100 - 125	4	18	3,400
	125 - 150	3	15	2,200
	150 - 200	2	17	2,500
	200 - 250	1	12	1,800
	250 - 500	2	15	1,800
	500 -1000	1	4	800
	1000 -2000	0	1	100
*Approximate Total:-		100	1000	1000,000

* for this calculation, suitable total droplet numbers for each expiratory act were chosen which were of the same order as the observed totals recorded in table 4.

Table 18:- Calculated Percentages of Droplets in Each Size Group Which Will Contain One or More Organisms When the Secretion Atomised Contains 30,000,000, 1,000,000, 30,000 or 1000 Organisms per Milliliter.

	Droplet Diameter in Microns	30,000,000 Commensals per ml.	1,000,000 Pathogens per ml.	30,000 Pathogens per ml.	1000 Pathogens per ml.
Remain Airborne as Droplet Nuclei	1 - 2	0.0059%	0.00020%	0.0000059%	0.00000020%
	2 - 4	0.047%	0.0016%	0.000047%	0.0000016%
	4 - 8	0.38%	0.013%	0.00038%	0.000013%
	8 - 16	3.0%	0.10%	0.0030%	0.00010%
	16 - 24	12%	0.44%	0.013%	0.00044%
	24 - 32	30%	1.2%	0.035%	0.0012%
	32 - 40	51%	2.4%	0.072%	0.0024%
	40 - 50	76%	4.8%	0.14%	0.0048%
	50 - 75	98%	12%	0.40%	0.013%
	75 - 100	100%	30%	1.1%	0.036%
Fall at Once to Ground	100 - 125	100%	53%	2.2%	0.075%
	125 - 150	100%	74%	4.1%	0.14%
	150 - 200	100%	95%	8.6%	0.29%
	200 - 250	100%	100%	16%	0.60%
	250 - 500	100%	100%	60%	3.1%
	500 - 1000	100%	100%	100%	22%
	1000 - 2000	100%	100%	100%	86%

Table 19:- Average Numbers of the Droplets Expelled by Expiratory Activities Which Are Likely to Contain Pathogenic or Commensal Organisms as Calculated from Figures in Tables 17 and 18.

Number of Organisms per Milliliter of Respiratory Secretions Atomised		Type of Droplet	SPEAKING LOUDLY 100 WORDS	ONE COUGH	ONE SNEEZE
Pathogens	1,000	Under 100 Microns	0 (0.003%)	0 (0.002%)	6 (0.0006%)
		All Sizes	0.3 (0.3%)	2 (0.2%)	350 (0.035%)
	30,000	Under 100 Microns	0.1 (0.1%)	0.6 (0.06%)	190 (0.019%)
		All Sizes	3 (3%)	19 (1.9%)	2,800 (0.28%)
	1,000,000	Under 100 Microns	3 (3%)	19 (1.9%)	5,600 (0.56%)
		All Sizes	13 (13%)	88 (8.8%)	16,000 (1.6%)
Commensals	30,000,000	Under 100 Microns	21 (21%)	170 (17%)	65,000 (6.5%)
		All Sizes	34 (34%)	250 (25%)	78,000 (7.8%)
Total Number of Droplets Containing and Not Containing Organisms.		Under 100 Microns	87 (87%)	920 (92%)	990,000 (99%)
		All Sizes	100 (100%)	1000 (100%)	1,000,000 (100%)

(Note:- the figure "0" is used for all average values less than 0.1; the numbers containing organisms are shown, in brackets, as percentages of the total numbers.)

Table 20:- Number of Commensal Bacteria in Droplets of Each Size Caught on Glass Slides Exposed at 6 Inches in front of the Mouth during Simulated Sneezing.

Measured Droplet Mark Diameter in Microns	Calculated Dimensions of Spherical Parent Droplets		Number of Droplets Examined	Number of Bacteria Seen in Each Droplet	Average Number per Droplet	Average Number per Ml.
	Diameter in Microns	Mean Volume in Cubic Microns				
6 - 15	2 - 5	27	22	0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,1,	0.1	37×10^8
15 - 30	5 - 10	246	66	0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,2,2,2,2,2,2,2,2,3,3,3,3,3,4,10,	0.9	37×10^8
30 - 45	10 - 15	1060	48	0,0,0,0,0,0,0,0,1,1,1,1,1,1,1,1,1,2,2,2,2,2,2,2,2,2,2,2,2,3,4,4,4,4,5,5,5,5,5,5,6,6,7,10,12,12,20,20,	3.6	34×10^8
45 - 60	15 - 20	2870	54	0,0,0,0,0,0,1,1,1,2,2,2,2,2,3,3,3,3,3,3,3,4,4,4,4,5,5,5,5,5,6,6,6,6,6,6,7,7,8,8,8,8,9,9,10,10,10,12,13,13,15,16,18,20,	5.8	20×10^8
60 - 90	20 - 30	8500	29	0,0,2,2,2,3,4,5,5,5,9,9,10,10,10,10,11,12,14,14,15,20,20,25,25,38,50,200,400,	32	38×10^8
90 - 120	30 - 40	22,900	15	2,6,8,12,20,21,25,30,30,30,43,46,47,90,200,	41	18×10^8
120 - 180	40 - 60	68,000	15	30,31,46,50,87,98,103,133,150,150,160,250,260,300,2000,	257	38×10^8
180 - 300	60 - 100	285,000	3	200,620,3000,	1270	44×10^8
300 - 450	100 - 150	1060,000	4	600,650,1000,1200,	860	8×10^8

Diameter of spherical parent droplet calculated as one-third of diameter of the droplet mark.

Table 21:- Number of Bacteria-Carrying Droplet Nuclei per Cubic Foot of Air in a 1700 Cu.Ft. Chamber at Various Times after its Contamination by One Simulated Sneeze as Calculated from Colony Counts Made on Blood Agar Plates Exposed in the Slit Sampler.

Time in Minutes after Sneezing	5 Experiments with Fan Run Only During First Minute					3 Experiments with Fan Run Throughout		
Control:-	3#	4#	5#	1#	6#	3#	2#	1#
½ - 1	403	471	83	-	294	401	76	82
2 - 2½	347	324	71	89	-	271	59	57
4½ - 5	276	312	45	71	118	159	40	50
7 - 7½	235	235	50	-	-	149	-	-
9 - 10	188	224	36	48	-	65	31	38
14 - 15	124	153	29	43	-	42	-	28
19 - 20	88	129	31	33	-	27	-	24
24 - 25	-	100	16	30	-	-	6	10
29 - 30	47	101	11	19	-	12	-	8
39 - 40	31	59	6	-	25	3	2	-
49 - 50	8	37	4	-	-	1	-	-
59 - 60	5	-	3	-	3	1	2	-

means Strept.viridans not present.

Table 22:- Disappearance of Bacteria-Carrying Droplet Nuclei Produced by Sneezing from Air of 1700 Cu.Ft. Chamber: Showing for Different Time Intervals Following Sneezing the Numbers Remaining Airborne Expressed as Percentages of the Numbers Present at ½ to 1 Minute after Sneezing.

(Calculated from counts per cubic foot of air given in table 21)

Time in Minutes after Sneezing	Fan Run Only During First Minute						Fan Run Throughout			
	5 Experiments					Average	3 Experiments			Average
Control:-	1	1	6	1	2	2	1	3	1	2
½ - 1	100	100	100	-	100	100	100	100	100	100
2 - 2½	86	69	86	100	-	85	68	78	70	72
4½ - 5	68	66	54	80	40	62	40	53	61	51
7 - 7½	57	50	60	-	-	56	37	-	-	37
9 - 10	47	48	43	54	-	48	16	41	46	34
14 - 15	31	33	35	48	-	37	10	-	34	22
19 - 20	22	27	37	37	-	31	7	-	29	18
24 - 25	-	21	19	34	-	25	-	8	12	10
29 - 30	12	21	13	21	-	17	3	-	10	7
39 - 40	8	13	7	-	9	9	1	3	-	2
49 - 50	2	8	5	-	-	5	¼	-	-	¼
59 - 60	1	-	4	-	-	3	¼	3	-	2
Disappearance shown in terms of Equivalent Ventilation Rate in overturns per hour.										
0 - 15	4.8	4.5	4.2	2.9			9.0	5.4	4.2	
15 - 30	3.9	1.7	3.9	3.3			5.0	6.5	5.1	
30 - 45	3.4	2.9	3.1	-	(3.6)	3.5	7.2	4.4	-	5.5
45 - 60	5.5	-	2.0	-			2.8	-	-	
Disappearance shown in terms of Equivalent Settling Rate in meters per second.										
0 - 15	0.0018	0.0018	0.0018	0.0014						
15 - 30	0.0016	0.0010	0.0016	0.0015		0.0015				
30 - 45	0.0015	0.0013	0.0013	-						
45 - 60	0.0021	-	0.0008	-						

Table 23:- Number of Bacteria-Carrying Droplet Nuclei per Cubic Foot of Air in a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze as Calculated from Colony Counts Made on Blood Agar Plates Exposed in the Slit Sampler.

Time in Minutes after Sneezing	5 Experiments with Fan Run Only During First Minute					3 Experiments with Fan Run Throughout		
Control:-	7#	5#	2#	7#	9#	4#	2#	5#
½ - 1	436	132	448	724	102	168	218	72
4½ - 5	265	85	-	399	67	70	82	34#
9½ - 10	201	46	282	343	40	11	21	38#
19 - 20	128	36	168	214	23	7	8	-
29 - 30	124	24	82	93	26	3#	6	-
44 - 45	73	-	34	50	12	2#	4#	-
59 - 60	46	11	21	29	8	-	5#	-
74 - 75	-	10	14	-	-	-	-	-
89 - 90	-	8	11	-	-	-	-	-
119 -120	-	7#	2	-	-	-	-	-

means Strept.viridans not present.

Table 24:- Disappearance of Bacteria-Carrying Droplet Nuclei Produced by Sneezing from Air of 70 Cu.Ft.Chamber: Showing for Different Time Intervals Following Sneezing the Number Remaining Airborne Expressed as Percentages of the Numbers Present at ½ to 1 Minute after Sneezing.

(Calculated from counts per cubic foot of air given in Table 23)

Time in Minutes after Sneezing	Fan Run Only During First Minute						Fan Run Throughout			
	5 Experiments					Average	3 Experiments			Average
Control:-	2	4	½	1	9	3	2	1	7	3
½ - 1	100	100	100	100	100	100	100	100	100	100
4½ - 5	61	64	-	55	66	62	42	38	47	43
9½ - 10	46	35	63	47	39	46	7	10	53	23
19 - 20	29	27	37	30	23	29	4	4	-	4
29 - 30	28	18	18	13	25	20	2	3	-	3
44 - 45	17	-	8	7	12	11	1	2	-	2
59 - 60	11	8	5	4	8	7	-	2	-	2
74 - 75	-	8	3	-	-	5	-	-	-	-
89 - 90	-	6	2	-	-	4	-	-	-	-
119 -120	-	5	½	-	-	3	-	-	-	-
	Disappearance shown in terms of Equivalent Ventilation Rate in overturns per hour.									
0 - 15	3.9	4.7	2.8	3.9	4.8		11.8	11.0	-	
15 - 30	1.2	2.1	4.0	4.3	0.7	2.7	-	-	-	11.4
30 - 45	2.1	1.6	3.5	2.5	3.0		-	-	-	
45 - 60	1.8	1.6	1.9	2.2	1.7		-	-	-	
	Disappearance shown in terms of Equivalent Settling Rate in meters per second.									
0 - 15	0.0012	0.0013	0.00095	0.0012	0.0013					
15 - 30	0.00044	0.00057	0.0012	0.0013	0.00038	0.00086				
30 - 45	0.00074	0.00050	0.0010	0.00087	0.00098					
45 - 60	0.00067	0.00050	0.00071	0.00081	0.00062					

Table 25:- Number of Bacteria-Carrying Droplet Nuclei per Cubic Foot of Air in a 70 Cu.Ft. Chamber at Various Times after its Contamination by 30 'Tongue-teeth Coughs' as Calculated from Colony Counts Made on Blood Agar Plates Exposed in the Slit Sampler.

Time in Minutes after Coughing	2 Experiments with Fan Run Only During First Minute		Average Percentages
Control:-	11#	8#	4%
½ - 1	245	212	100%
9½ - 10	156	58	45%
19 - 20	88	-	36%
29 - 30	18	22	9%
59 - 60	12	6	4%

means Strept.viridans not present.

Table 26:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.05 Cu.Ft. of Air of a 1700 Cu.Ft. Chamber at Various Times after its Contamination by One Simulated Sneeze.

Fan only for first minute	Droplet Nucleus Diameter in Microns ½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	Total of All Sizes
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	324	51	25	8	5	2	1	1	0	1		418
2 - 2½	331	74	27	6	5	3	0	1	1	0		448
4½ - 5	287	85	19	8	4	1	0	0	0	0		404
7 - 7½	276	44	11	3	2	0	0	0	0	0		336
9½ - 10	223	38	11	4	1	0	0	0	0	0		277
14 - 15	149	32	4	1	0	0	0	0	0	0		186
29 - 30	71	16	1	1	0	0	0	0	0	0		89
44 - 45	36	8	0	0	0	0	0	0	0	0		44
59 - 60	20	5	0	0	0	0	0	0	0	0		25
74 - 75	14	4	0	0	0	0	0	0	0	0		18

Time in Minutes after Sneezing

Table 27:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.05 Cu.Ft. of Air of a 1700 Cu.Ft. Chamber at Various Times after its Contamination by One Simulated Sneeze.

Fan only for first minute	Droplet Nucleus Diameter in Microns										Total of All Sizes
Time in Minutes after Sneezing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	177	50	19	4	2	1	0	0	0	0	0	253
2 - 2½	144	38	12	3	2	0	0	0	0	0	0	199
9½ - 10	56	26	7	2	1	0	0	0	0	0	0	92
19 - 20	49	26	4	2	0	0	0	0	0	0	0	81
29 - 30	29	7	1	0	0	0	0	0	0	0	0	37
44 - 45	28	6	0	0	0	0	0	0	0	0	0	34
59 - 60	25	5	0	0	0	0	0	0	0	0	0	30

Table 28:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.05 Cu.Ft. of Air of a 1700 Cu.Ft. Chamber at Various Times after its Contamination by One Simulated Sneeze.

Fan only for first minute	Droplet Nucleus Diameter in Microns										Total of All Sizes
Time in Minutes after Sneezing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	102	44	19	12	4	2	1	0	0	0		184
2 - 2½	74	75	20	8	3	2	1	1	1	0		185
4½ - 5	56	47	19	5	2	0	0	0	0	0		129
7 - 7½	32	29	9	4	2	1	0	0	0	0		77
9½ - 10	27	21	6	2	1	0	0	0	0	0		57
14 - 15	20	25	6	2	1	0	0	0	0	0		54
29 - 30	6	5	1	0	0	0	0	0	0	0		12
44 - 45	3	3	0	0	0	0	0	0	0	0		6

Table 29:- Disappearance of Congo Red-Containing Droplet Nuclei Produced by Sneezing from Air of 1700 Cu.Ft. Chamber: Showing for Different Time Intervals Following Sneezing the Numbers Remaining Airborne Expressed as Percentages of the Numbers Present at ½ to 1 Minute after Sneezing.

(Calculated from counts per 0.05 Cu.Ft. of air given in tables 26, 27 and 28)

Time In Minutes after Sneezing	3 Experiments with Fan Run Only During First Minute (from Table 26)	(from Table 27)	(from Table 28)	Average
½ - 1	100	100	100	100
2 - 2½	107	79	101	96
4½ - 5	96	-	70	83
7 - 7½	80	-	42	61
9½ - 10	66	36	31	44
14 - 15	44	-	29	37
19 - 20	-	32	-	32
29 - 30	21	15	7	14
44 - 45	11	13	3	9
59 - 60	6	12	-	9
74 - 75	4	-	-	4
	The Disappearance shown in terms of Equivalent Ventilation Rate in overturns per hour.			
0 - 15	3.3	4.3	5.0	
15 - 30	2.9	3.2	5.7	3.4
30 - 45	2.6	0.6	3.3	
	The Disappearance shown in terms of Equivalent Settling Rate in meters per second.			
0 - 15	0.0015	0.0018	0.0019	
15 - 30	0.0013	0.0015	0.0021	0.0015
30 - 45	0.0013	0.00035	0.0015	

Table 30:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

Fan only for first minute.	Droplet Nucleus Diameter in Microns											Total of All Sizes	Carrying Bacteria
Time in Minutes after Sneezing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20		
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.2#
½ - 1	89	228	129	37	20	9	5	2	1	1	1	522	16.1
9½- 10	79	181	107	23	6	1	0	0	0	0	0	397	6.6
19 - 20	56	130	68	14	2	0	0	0	0	0	0	270	3.0
29 - 30	52	124	33	12	1	0	0	0	0	0	0	222	1.5
59 - 60	42	100	32	5	0	0	0	0	0	0	0	179	0.7
74 - 75	29	64	16	1	0	0	0	0	0	0	0	110	0.6
89 - 90	27	60	15	0	0	0	0	0	0	0	0	102	0.3
119 -120	32	65	12	0	0	0	0	0	0	0	0	109	0.3#
149 -150	27	44	13	0	0	0	0	0	0	0	0	84	-
359 -360	23	21	2	0	0	0	0	0	0	0	0	46	0.3#
10 hours	8	2	0	0	0	0	0	0	0	0	0	10	-
30 hours	4	1	0	0	0	0	0	0	0	0	0	5	-

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 31:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

Fan only for first minute.	Droplet Nucleus Diameter in Microns ½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	Total of All Sizes.	Carrying Bacteria
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.3#
½ - 1	82	430	249	102	39	19	7	2	0	1	0	931	68.2
3 - 3½	86	437	210	64	19	6	1	0	0	0	0	823	-
4½- 5	112	393	148	62	12	2	0	0	0	0	0	729	-
9½- 10	93	357	188	44	10	2	0	0	0	0	0	694	36.8
19 - 20	116	269	154	24	3	0	0	0	0	0	0	566	22.8
29 - 30	145	260	78	10	2	0	0	0	0	0	0	495	14.4
44 - 45	104	211	74	5	1	0	0	0	0	0	0	395	10.3
59 - 60	102	192	28	5	0	0	0	0	0	0	0	327	7.0
74 - 75	47	109	27	0	0	0	0	0	0	0	0	183	3.0
89 - 90	81	124	34	0	0	0	0	0	0	0	0	239	2.4
119 -120	78	102	7	2	0	0	0	0	0	0	0	189	1.1
179 -180	24	28	4	0	0	0	0	0	0	0	0	56	-
239 -240	36	62	9	0	0	0	0	0	0	0	0	107	0.1#
299 -300	31	21	1	0	0	0	0	0	0	0	0	53	-
359 -360	20	11	2	0	0	0	0	0	0	0	0	33	-
659 -660	3	2	0	0	0	0	0	0	0	0	0	5	-
24 hours	0	0	0	0	0	0	0	0	0	0	0	0	-

Time in Minutes after Sneezing

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 32:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

Fan only for first minute.	Droplet Nucleus Diameter in Microns											Total of All Sizes.	Carrying Bacteria
Time in Minutes after Sneezing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20		
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.2#
½ - 1	43	93	78	26	9	4	2	1	1	0	0	257	6.4
2 - 2½	58	88	66	22	8	5	0	1	0	0	0	248	-
9½- 10	79	86	34	17	3	1	0	0	0	0	0	220	2.0
19½- 20	40	35	19	3	1	0	0	0	0	0	0	98	0.9
29½- 30	36	38	11	4	1	0	0	0	0	0	0	90	0.5
44 - 45	16	21	3	1	0	0	0	0	0	0	0	41	0.4
59 - 60	21	19	7	1	0	0	0	0	0	0	0	48	0.2
89 - 90	18	11	3	0	0	0	0	0	0	0	0	32	0.1#
119 -120	9	11	3	0	0	0	0	0	0	0	0	23	0.1#
149 -150	8	8	1	0	0	0	0	0	0	0	0	17	0.1#
5 hours	4	2	0	0	0	0	0	0	0	0	0	6	0.2#

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 33:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

Fan only for first minute. Time in Minutes after Sneezing.	Droplet Nucleus Diameter in Microns ½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	Total of All Sizes	Carrying Bacteria
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.1#
½ - 1	57	357	260	59	26	8	1	1	0	1	0	770	9.2
4 - 5	20	342	149	49	12	3	1	1	0	0	0	557	4.4
9 - 10	27	300	158	40	9	2	1	0	0	0	0	537	3.6
19 - 20	29	331	151	22	8	2	0	0	0	0	0	543	1.4
29 - 30	25	231	80	13	2	0	0	0	0	0	0	351	1.2
44 - 45	34	180	79	14	1	0	0	0	0	0	0	308	0.7
59 - 60	20	186	66	3	0	0	0	0	0	0	0	275	0.2
4 hours	15	89	5	0	0	0	0	0	0	0	0	109	0.3

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 34:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

Fan only for first minute.	Droplet Nucleus Diameter in Microns											Total of All Sizes.	Carrying Bacteria
Time in Minutes after Sneezing.	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20		
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.2#
½ - 1	2	22	23	8	4	2	1	1	0	0	0	63	5.2
4 - 5	3	27	27	6	3	1	0	0	0	0	0	67	1.7
9 - 10	2	29	27	4	1	0	0	0	0	0	0	63	1.0
19 - 20	1	16	15	4	1	0	0	0	0	0	0	37	0.5
44 - 45	1	9	11	1	0	0	0	0	0	0	0	22	0.1

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 35:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

Fan only for first minute.	Droplet Nucleus Diameter in Microns ½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	Total of All Sizes	Carrying Bacteria
Time in Minutes after Sneezing: Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.2#
½ - 1	0	36	63	24	7	3	1	1	1	0	0	136	16.9
9 - 10	1	35	59	8	3	1	0	0	0	0	0	107	7.1
29 - 30	0	44	37	2	1	0	0	0	0	0	0	84	1.7
119 -120	0	23	9	1	0	0	0	0	0	0	0	33	0.2#
179 -180	0	22	17	0	0	0	0	0	0	0	0	39	0.2#
6 hours	0	10	8	0	0	0	0	0	0	0	0	18	-

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 36:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

FAN RUN THROUGHOUT	Droplet Nucleus Diameter in Microns											Total of All Sizes	Carrying Bacteria
Time in Minutes after Sneezing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20		
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.2#
½ - 1	136	224	134	46	18	8	2	1	0	1	0	570	22.3
2 - 2½	120	222	121	18	6	2	0	0	0	0	0	489	-
4½- 5	94	162	66	12	2	1	0	0	0	0	0	337	4.8
9½- 10	68	78	28	0	0	0	0	0	0	0	0	174	1.7
19½- 20	11	16	4	0	0	0	0	0	0	0	0	31	0.4
29½- 30	6	9	0	0	0	0	0	0	0	0	0	15	0.2
44 - 45	2	1	0	0	0	0	0	0	0	0	0	3	0.1#
59 - 60	0	0	0	0	0	0	0	0	0	0	0	0	0.3#

Bacteria-carrying nuclei counted by subsequent sampling on blood agar. # means no Strept.viridans.

Table 37:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.02 Cu.Ft. of the Air of a 70 Cu.Ft. Chamber at Various Times after its Contamination by One Natural Sneeze.

FAN RUN THROUGHOUT	Droplet Nucleus Diameter in Microns											Total of All Sizes	Carrying Bacteria
Time in Minutes after Sneezing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20		
Control:-	0	0	0	0	0	0	0	0	0	0	0	0	0.2#
½ - 1	28	79	67	28	8	4	1	0	0	0	0	215	15.7
2 - 2½	16	67	46	16	4	1	0	0	0	0	0	150	-
4½- 5	15	61	40	5	1	0	0	0	0	0	0	122	3.0
9½- 10	10	32	11	2	0	0	0	0	0	0	0	55	0.8
19½- 20	1	0	0	0	0	0	0	0	0	0	0	1	0.4

Bacteria-carrying nuclei counted by subsequently sampling on blood agar. # means no Strept.viridans.

Table 38:- Disappearance of Congo Red-Containing Droplet Nuclei Produced by Sneezing from Air of a 70 Cu.Ft. Chamber: Showing for Different Time Intervals Following Sneezing the Numbers Remaining Airborne Expressed as Percentages of the Numbers Present at ½ to 1 Minute after Sneezing.

(Calculated from counts per 0.02 Cu.Ft. of air given in tables 30-37)

Time in Minutes after Sneezing	6 Experiments with Fan Run Only During First Minute							2 Experiments with Fan Run Throughout		
	(tab.30)	(tab.31)	(tab.32)	(tab.33)	(tab.34)	(tab.35)	Average	(tab.36)	(tab.37)	Average
½ - 1	100	100	100	100	100	100	100	100	100	100
2 - 2½	-	87	97	-	-	-	92	86	70	78
4½ - 5	-	78	-	72	106	-	85	59	57	58
9½ - 10	76	75	86	70	100	79	81	31	26	29
19 - 20	52	60	38	71	59	-	56	5	½	3
29 - 30	43	53	35	46	-	62	48	3	-	3
44 - 45	-	42	16	40	35	-	33	½	-	½
59 - 60	34	35	19	36	-	-	31	0	-	0
74 - 75	21	20	-	-	-	-	21	-	-	-
89 - 90	20	26	12	-	-	-	19	-	-	-
119 -120	21	20	9	-	-	24	19	-	-	-
149 -150	16	-	7	-	-	-	12	-	-	-
179 -180	-	6	-	-	-	29	18	-	-	-
239 -240	-	11	-	14	-	-	13	-	-	-
299 -300	-	6	2	-	-	-	4	-	-	-
359 -360	9	4	-	-	-	-	7	-	-	-
559 -600	2	-	-	-	-	-	2	-	-	-
659 -660	-	½	-	-	-	13	7	-	-	-
24-30 hours	1	0	-	-	-	-	½	-	-	-
Disappearance shown in terms of Equivalent Ventilation Rate in overturns per hour.										
0 - 15	1.8	1.6	1.8	1.4	0.9	(1.0)		6.8	8.2	
15 - 30	1.6	1.0	2.4	1.7	2.1			7.3	-	
30 - 45	0.5	0.9	(1.2)	0.6	1.2	-	1.2	-	-	7.4
45 - 60	0.5	0.7		0.4	-	-		-	-	
Disappearance shown in terms of Equivalent Settling Rate in meters per second.										
0 - 15	0.00068	0.00062	0.00072	0.00057	0.00038	(0.00036)				
15 - 30	0.00062	0.00040	0.00083	0.00064	0.00077					
30 - 45	0.00020	0.00032	(0.00043)	0.00025	0.00047	-	0.00046			
45 - 60	0.00020	0.00032		0.00019	-	-				

Table 39:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.3 Cu.Ft. of Air of a 70 Cu.Ft. Chamber at Various Times after Contamination by 20 'Tongue-teeth Coughs'.

Fan only for first minute	Droplet Nucleus Diameter in Microns											Total of All Sizes
Time in Minutes after Coughing	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	0	11	61	34	16	12	7	2	1	1	0	145
9 - 10	0	12	53	18	13	2	0	0	0	0	0	98
19 - 20	0	7	43	20	0	0	0	0	0	0	0	70
29 - 30	0	10	29	11	0	0	0	0	0	0	0	50
59 - 60	0	8	13	2	0	0	0	0	0	0	0	23
74 - 75	0	6	10	0	0	0	0	0	0	0	0	16

Table 40:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.3 Cu.Ft. of Air of a 70 Cu.Ft. Chamber at Various Times after Contamination by 20 'Tongue-teeth Coughs'.

Time in Minutes after Coughing (Fan only for first minute)	Droplet Nucleus Diameter in Microns ½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	Total of All Sizes
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	18	223	380	188	120	96	30	18	0	0	0	1073
2 - 2½	6	100	281	153	47	12	5	0	0	0	0	604
9 - 10	5	98	123	32	11	7	0	0	0	0	0	276
19 - 20	17	65	103	34	6	0	0	0	0	0	0	225
29 - 30	8	78	110	25	2	0	0	0	0	0	0	223

Table 41:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.3 Cu.Ft. of Air of a 70 Cu.Ft. Chamber at Various Times after Contamination by 20 'Tongue-teeth Coughs'.

Fan only for first minute	Droplet Nucleus Diameter in Microns											Total of All Sizes
Time in Minutes after Coughing.	½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	0	7	92	50	23	13	8	7	3	1	2	206
9 - 10	0	10	54	19	9	1	0	0	0	0	0	93
29 - 30	0	6	46	15	1	0	0	0	0	0	0	68
89 - 90	0	8	20	1	0	0	0	0	0	0	0	29
179 -180	0	6	7	0	0	0	0	0	0	0	0	13

Table 42:- Number of Congo Red-Containing Droplet Nuclei of Each Size Counted Microscopically per 0.3 Cu.Ft. of Air of a 70 Cu.Ft. Chamber at Various Times after Contamination by 20 'Tongue-teeth Coughs'.

Time in Minutes after Coughing (Fan only for first minute)	Droplet Nucleus Diameter in Microns ½-1	1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	Total of All Sizes
Control:-	0	0	0	0	0	0	0	0	0	0	0	0
½ - 1	0	3	66	34	22	18	5	4	5	3	3	163
9 - 10	0	3	29	13	8	3	0	1	0	0	0	57
29 - 30	0	2	21	4	0	0	0	0	0	0	0	27

Table 43:- Disappearance of Congo Red-Containing Droplet Nuclei Produced by Coughing from Air of a 70 Cu.Ft. Chamber: Showing for Different Time Intervals Following Coughing the Numbers Remaining Airborne Expressed as Percentages of the Numbers Present at ½ to 1 Minute after Coughing.

(Calculated from counts per 0.3 Cu.Ft. of air given in tables 39-42)

Time in Minutes after Coughing	4 Experiments with Fan Run Only During First Minute				Average
	(table 39)	(table 40)	(table 41)	(table 42)	
½ - 1	100	100	100	100	100
2 - 2½	-	56	-	-	56
9½ - 10	68	26	45	35	44
19 - 20	48	21	-	-	35
29 - 30	35	21	33	15	26
59 - 60	16	-	-	-	16
74 - 75	11	-	-	-	11
89 - 90	-	-	14	-	14
179 -180	-	-	6	-	6

Table 44:- Time in Minutes for 90% Reduction (i.e. to 10%) of Droplet Nucleus Air Contamination by Sedimentation and other Natural Processes in a Closed Chamber.

(times taken from data in tables 22,24,25,29,38 and 43)

Fan	Expiratory Act	Chamber Volume	Time in Minutes for 90% Reduction of Air Infection	
			Bacteria-carrying Nuclei	All Microscopically Visible Nuclei
Run for first minute only	Sneezing	70 Cu.Ft.	30 - 60 (av. 45)	120 - 660+ (av. 300)
		1700 Cu.Ft.	30 - 50 (av. 40)	20 - 60+ (av. 45)
	Coughing	70 Cu.Ft.	30	75 - 180
Run throughout	Sneezing	70 Cu.Ft.	10 - 20	10 - 20
		1700 Cu.Ft.	15 - 30 (av. 25)	

Table 45:- Number of Congo Red-Containing Droplet Marks of Each Size on Glass Slides Exposed at 5 Feet below Mouth at Different Times after a Simulated Sneeze and the Calculated Average Sedimentation Rates of these Droplets.

Diameter in Microns	Slide Exposure Time in Seconds after Sneeze							
	0-2.	2-4.	4-6.	6-8.	8-10.	10-12.	12-14.	14-16
25 - 50	5'	0	0	0	0	1	1'	0
50 - 75	4	0	0	0	1	6'	8'	0
75 - 100	1	0	0	1	2	3'	5'	4
100- 150	1	1	3	3	39	22'	4	0
150- 200	1	1	3	11	6	4	0	0
200- 300	1	8	20	18	0	0	0	0
300- 400	1	12	2	0	0	0	0	0
400- 500	0	6	0	0	0	0	0	0
500- 600	14	0	0	0	0	0	0	0
600- 700	10	0	0	0	0	0	0	0
700- 800	6	0	0	0	0	0	0	0
800-1000	3	0	0	0	0	0	0	0
1000-1500	4	0	0	0	0	0	0	0
1500-3000	0	0	0	0	0	0	0	0
3000-5000	0	0	0	0	0	0	0	0
5000-7000	1	0	0	0	0	0	0	0
All Sizes	52	28	28	33	48	36	18	4
Scan Area in Sq.Mm.	43	36	30	64	62	56	115	193
Number per 43 Sq.Mm.	52	33	40	22	33	28	7	1
Settling Rate in Meters per Second	1.7-0.85 #	0.85-0.43	0.43-0.28	0.28-0.21	0.21-0.17	0.17-0.14	0.14-0.12	0.12-0.11

' numbers marked thus (5') indicate droplets of which some were largely evaporated before reaching slide.

one second was taken as the shortest settling time.

Table 46:- Number of Congo Red-Containing Droplet Marks of Each Size on Glass Slides Exposed to the Sedimenting Droplets at 5 Feet below the Mouth at Different Times after a Simulated Sneeze. and Calculated Average Sedimentation Rates.

Diameter in Microns	Slide Exposure Time in Seconds after Sneeze						
	0-2	2-4	4-6	6-8	8-10	10-12	12-14
25 - 50	0	0	0	0	1	0	0
50 - 75	0	0	0	0	1	1	0
75 - 100	0	0	0	6	1	1	0
100 - 150	0	0	3	9	5	2	0
150 - 200	0	0	8	6	8	0	0
200 - 300	0	8	20	7	3	0	0
300 - 400	0	12	2	0	0	0	0
400 - 500	2	4	0	0	0	0	0
500 - 600	5	0	0	0	0	0	0
600 - 700	2	0	0	0	0	0	0
700 - 800	0	0	0	0	0	0	0
800 -1000	0	0	0	0	0	0	0
1000-1500	1	0	0	0	0	0	0
1500-more	0	0	0	0	0	0	0
All Sizes	10	24	33	28	19	4	0
Scan Area in Sq.Mm.	39	23	24	50	103	86	172
Number per 43 Sq.Mm.	11	45	60	24	8	2	0
Settling Rate in Meters per Second	1.7-0.85	0.85-0.43	0.43-0.28	0.28-0.21	0.21-0.17	0.17-0.14	0.14-0.12

Table 47:- Number of Dye-Containing Droplet Nuclei of Each Size Sedimented on to Slides Exposed on Floor after Simulated Sneezing from Standing Position.

Experiment:-	1	2	3	4
Dye Taken into Mouth:-	0.1 gm. Congo Red	0.1 gm. Congo Red	0.1 gm. Eosin	0.01 gm. Eosin
Expiratory Activity:-	1 Simulated Sneeze	1 Simulated Sneeze	10 Simulated Sneezes	10 Simulated Sneezes
Chamber:-	100 Cu.Ft.	1700 Cu.Ft.	100 Cu.Ft.	100 Cu.Ft.
Time of Exposure of Slides after Sneezing:-	From 1 to 60 Minutes	From 0 to 60 Minutes	From 1 Minute to 18 Hours	From 0 Minutes to 18 Hours
Nucleus Diameter in Microns: ¼ - ½	0	0	0	0
½ - 1	0	0	15	1
1 - 2	0	0	31	12
2 - 4	0	0	39	46
4 - 6	4	2	23	35
6 - 8	15	8	11	24
8 - 10	10	3	4	8
10 - 12	6	2	3	4
12 - 14	15	4	2	1
14 - 16	6	4	1	1
16 - 18	9	6	0	1
18 - 20	4	2	1	0
20 - 25	2	6	0	1
25 - 30	0	3	0	0
30 - 35	0	1	0	0
Maximum Diameter:-	23 Microns	35 Microns	19 Microns	22 Microns
Minimum Sedimentation Rate: Meters per Second.	0.0005	0.0005	0.000025	0.000025

Table 48:- Number of Congo Red-Containing Droplet Marks and Droplet Nuclei of Each Size Observed Microscopically on Glass Slides Exposed in Slit Sampler to about 1/60 Cu.Ft. of Air of a 2½ Cu.Ft. Chamber at Different Times after its Contamination by a Simulated Sneeze.

Diameter in Microns	Experiment 1, Sampling at 1-2 Seconds after Sneeze		Experiment 2, Sampling at 2-3 Seconds after Sneeze		Experiment 3, Sampling at 3-4 Seconds after Sneeze	
	Droplet Nuclei	Droplet Marks	Droplet Nuclei	Droplet Marks	Droplet Nuclei	Droplet Marks
¼ - ½	17000	0	0	0	19000	0
½ - 1	27000	0	0	0	10000	0
1 - 2	46000	0	10000	0	6500	0
2 - 4	21000	0	17000	0	6500	0
4 - 6	3400	1	3700	0	1500	0
6 - 8	170	3	510	1	650	0
8 - 10	0	5	170	1	200	0
10 - 12	0	0	0	1	40	0
12 - 14	0	3	0	2	10	1
14 - 16	0	1	0	2	8	1
16 - 18	0	1	0	2	1	2
18 - 20	0	5	0	2	0	1
20 - 25	0	3	0	2	0	2
25 - 30	0	4	0	1	0	2
30 - 40	0	11	0	2	0	5
40 - 50	0	10	0	0	0	3
50 - 75	0	6	0	4	0	5
75 -100	0	0	0	0	0	4
100-125	0	0	0	0	0	1
125-	0	0	0	0	0	0

All except the smallest numbers (under 10) given for the droplet nuclei, are not the actual counts made but are estimates based on counts of nuclei in a known fraction of the dust-line. The numbers given for the droplet marks are the actual counts for the whole dust-line.

Table 49:- Number of Congo Red-Containing Droplet Marks and Droplet Nuclei of Each Size Observed Microscopically on Glass Slides Exposed in Slit Samplers to about 1/60 Cu.Ft. of Air of a 1700 Cu.Ft. Chamber at Different Times after a Simulated was Delivered Towards the Two Samplers from Distance of 1½ Feet: Actual Counts for 1/300 Cu.Ft. of Air.

Diameter in Microns	Sampler A. Run at 0-1 Seconds after Sneeze		Sampler B. Run at 1-2 Seconds after Sneeze	
	Droplet Nuclei	Droplet Marks	Droplet Nuclei	Droplet Marks
¼ - ½	0	0	0	0
½ - 1	4	0	0	0
1 - 2	38	0	4	0
2 - 4	48	3	5	1
4 - 6	11	1	4	0
6 - 8	9	1	3	0
8 - 10	4	1	0	0
10 - 12	4	0	1	0
12 - 14	0	1	0	1
14 - 16	0	1	0	0
16 - 18	0	2	0	0
18 - 20	0	1	0	1
20 - 25	0	3	0	1
25 - 30	0	3	0	0
30 - 40	0	2	0	1
40 - 50	0	4	0	1
50 - 75	0	9	0	3
75 -100	0	1	0	0
100 -125	0	1	0	0
125 -	0	0	0	0

Both samplers were run in relation to the same sneeze.

Table 50:- Number of Congo Red-Containing Droplet Marks and Droplet Nuclei of Each Size Observed Microscopically on Glass Slides Exposed in Two Slit Samplers to about 1/60 Cu.Ft. of Air of a 1700 Cu.Ft. Chamber at Different Times after a Simulated Sneeze Was Delivered Towards the Samplers from Distance of 1½ Feet: Numbers for 1/600 Cu.Ft. of Air are Given.

Diameter in Microns	Sampler A. Run at 0-1 Seconds after Sneeze		Sampler B. Run at 1-2 Seconds after Sneeze	
	Droplet Nuclei	Droplet Marks	Droplet Nuclei	Droplet Marks
¼ - ½	0	0	0	0
½ - 1	50#	0	40#	0
1 - 2	340#	0	300#	0
2 - 4	400#	0	420#	0
4 - 6	150#	0	100#	0
6 - 8	16	1	20	0
8 - 10	2	0	7	1
10 - 12	2	0	2	0
12 - 14	0	1	1	0
14 - 16	0	0	0	0
16 - 18	0	1	0	2
18 - 20	0	1	0	0
20 - 25	0	1	0	1
25 - 30	0	1	0	0
30 - 40	0	3	0	0
40 - 50	0	1	0	0
50 - 75	0	2	0	2
75 -100	0	1	0	1
100 -125	0	1	0	1
125 -200	0	0	0	1

numbers marked thus are not actual counts, but are calculated from counts made on a known fraction of the part of the dust-line representing 1/600 Cu.Ft. which was scanned and counted for the marks and nuclei in other categories.

Both samplers were run in relation to same sneeze.

Table 51:- Number of Congo Red-Containing Droplet Marks and Droplet Nuclei of Each Size Observed Microscopically on Glass Slides Exposed in Slit Samplers to about 1/60 Cu.Ft. of Air of a 1700 Cu.Ft. Chamber at Different Times after a Simulated Sneeze Was Delivered Towards Samplers from Distance of 1½ Feet: Numbers for 1/60 Cu.Ft. of Air.

Diameter in Microns	Sampler A. Run at 1-2 Seconds after Sneeze		Sampler B. Run at 4-5 Seconds after Sneeze	
	Droplet Nuclei	Droplet Marks	Droplet Nuclei	Droplet Marks
¼ - ½	0	0	0	0
½ - 1	180#	0	25#	0
1 - 2	590#	0	140#	0
2 - 4	640#	0	150#	0
4 - 6	330#	0	90#	0
6 - 8	160#	2	75#	0
8 - 10	100#	2	50#	0
10 - 12	20	3	10	0
12 - 14	4	3	0	0
14 - 16	3	3	0	0
16 - 18	0	1	0	0
18 - 20	0	0	0	0
20 - 25	0	7	0	0
25 - 30	0	1	0	0
30 - 40	0	9	0	0
40 - 50	0	5	0	1
50 - 75	0	12	0	1
75 -100	0	9	0	1
100-125	0	5	0	0
125-200	0	6	0	0

numbers marked thus are not the actual counts, but are calculated from counts made on a known fraction of the dust-line.
Both samplers were run in relation to the same sneeze.

Table 52:- Evaporation Time of Droplets of Each Size Caught on Slides Exposed at 1 Foot in front of Mouth During a Simulated Sneeze as Measured by Microscopical Observation.

Approximate Droplet Mark Diameter in Microns	Calculated Diameter of Parent Spherical Droplet in Microns	EVAPORATION TIME IN SECONDS			
		Glass Slides		Celluloid Slides	
		Individual Droplets	Average	Individual Droplets	Average
150	50	20,20,12,10,10,5,5,5,5,	10	8,6,4,2,1,1,1,	3
300	100	55,15,12,7,5,	19	20,20,5,3,2,	10
600	200	60,40,15,	38	40,30,25,20,	29
1500	500	90,90,75,	85	65,	65
3000	1000	150,	150		

Temperature about 60°C. Relative Humidity about 60%.

Table 53:- Sedimentation Rates in Meters per Second of Differently-Sized Droplets and Nuclei as Observed by Various Methods in the Present Investigation Compared with the Rates Calculated from Stokes' Law by Wells (1934).

Particle.	Method of Observation	Values Observed in Present Investigation			Sedimentation Rate Calculated From Stokes' Law
		Diameter in Microns	Time to Fall 1.7 Meters	Sedimentation Rate	
Droplet	Naked-eye observation of large droplets falling to floor.	1000 and over	1 second	1.7	-
Droplet	Scanning slides exposed on floor at different times after sneezing (Tables 45,46).	200	1-2 seconds	0.85 - 1.7	1.3
		"100" #	2-4 seconds	0.43 - 0.85	0.3
Nucleus	Time till slide exposed in slit sampler is devoid of nuclei of this size (Tables 26-42).	20	2 minutes	0.014	0.012
		10	15 minutes	0.0019	0.003
		5	60 minutes	0.00047	0.0008
Nucleus	Smallest nucleus sedimented on to slides on floor in this time (Table 47).	5	60 minutes	0.00047	0.0008
		1	18 hours	0.000025	0.00003

100 microns was calculated diameter on reaching floor: diameter at start of fall, before evaporation, would have been appreciably greater.

Table 54:- Number of B.mesentericus-containing Droplet Nuclei per Cubic Foot of Air of a 100 Cu.Ft. Chamber at Various Times after 10 Simulated Sneezes Made with Mouth Containing a Heavy Suspension of Spores.

Time After Sneezing	Experiment 1	Experiment 2
(Before Sneezing):-	0	0
10 - 10½ min.	many	many
30 - 30½ min.	many	many
100 -100½ min.	6000	many
300 - 301 min.*	1600	224
500 - 504 min.*	176	-
1000-1005 min.*	0	0
Reinfection of air with B.mesentericus-containing nuclei while chamber disturbed by person marching in it for 10 minutes.		
1 day	0 to 59	0 to 37
5 days	-	0 to 78
7 days	0.2 to 43	0.7 to 12
9 days	0.3 to 29	-
11 days	0.2 to 37	-

* At these times, the presence or absence in the air of B.mesentericus as shown by the slit sampler was confirmed by sampling with a freshly introduced sieve plate sampler and a freshly introduced settling plate.

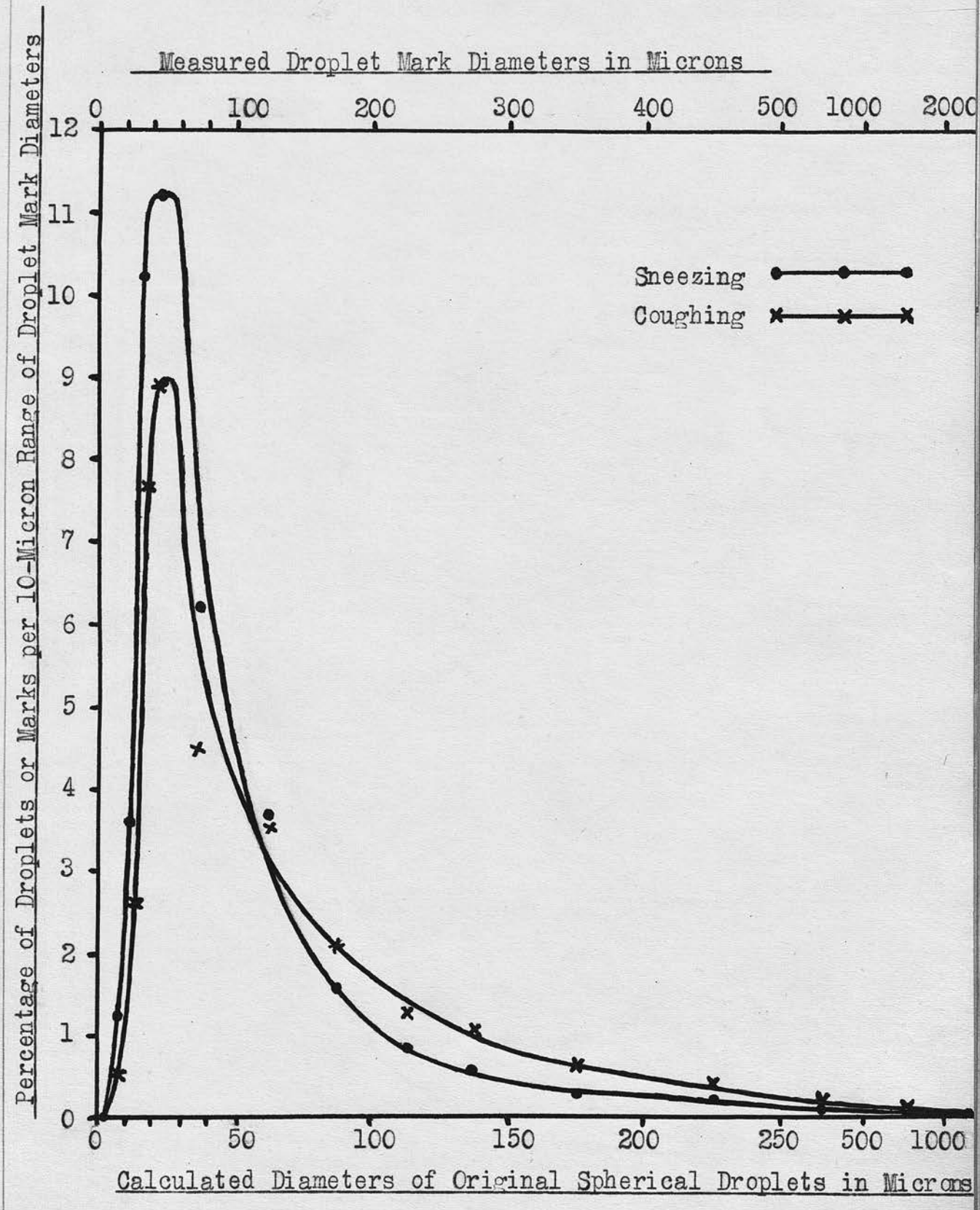
Table 55:- Droplet Evaporation Times in Seconds as Observed and Calculated by Different Methods.

Initial Droplet Diameter in Microns	Calculated by Wells (1934) for Water Droplets in Unsaturated Air at 18°C.	Averages of Times Observed by Each Method in Present Investigation					Approximate Overall Average of Observed Times
		By Microscopical Observation of Hanging Drops at about 16°C. and 60% R.H. (Table 13).			Mouth Spray (Saliva) Droplets on Slides at 16°C. & 60% R.H. (Table 52)	Calculated from Size of Largest Mouth Spray (Saliva) Nucleus Found in Air (Section 33)	
		Water Drops	Saliva Drops	Saliva & 1-2% Congo Red			
1000	165	1400	1300	1500	-	-	1200
500	41	380	360	-	80	-	300
200	6.6	-	-	-	32	-	48
100	1.7	-	-	-	15	10	12
50	0.4	-	-	-	7	1-2	3
Column	(1)	(2)	(3)	(4)	(5)	(6)	(7)

Column (7) shows the approximate averages of the values given in Columns 3-6, corrected so as to be proportional to the square of the droplet diameter.

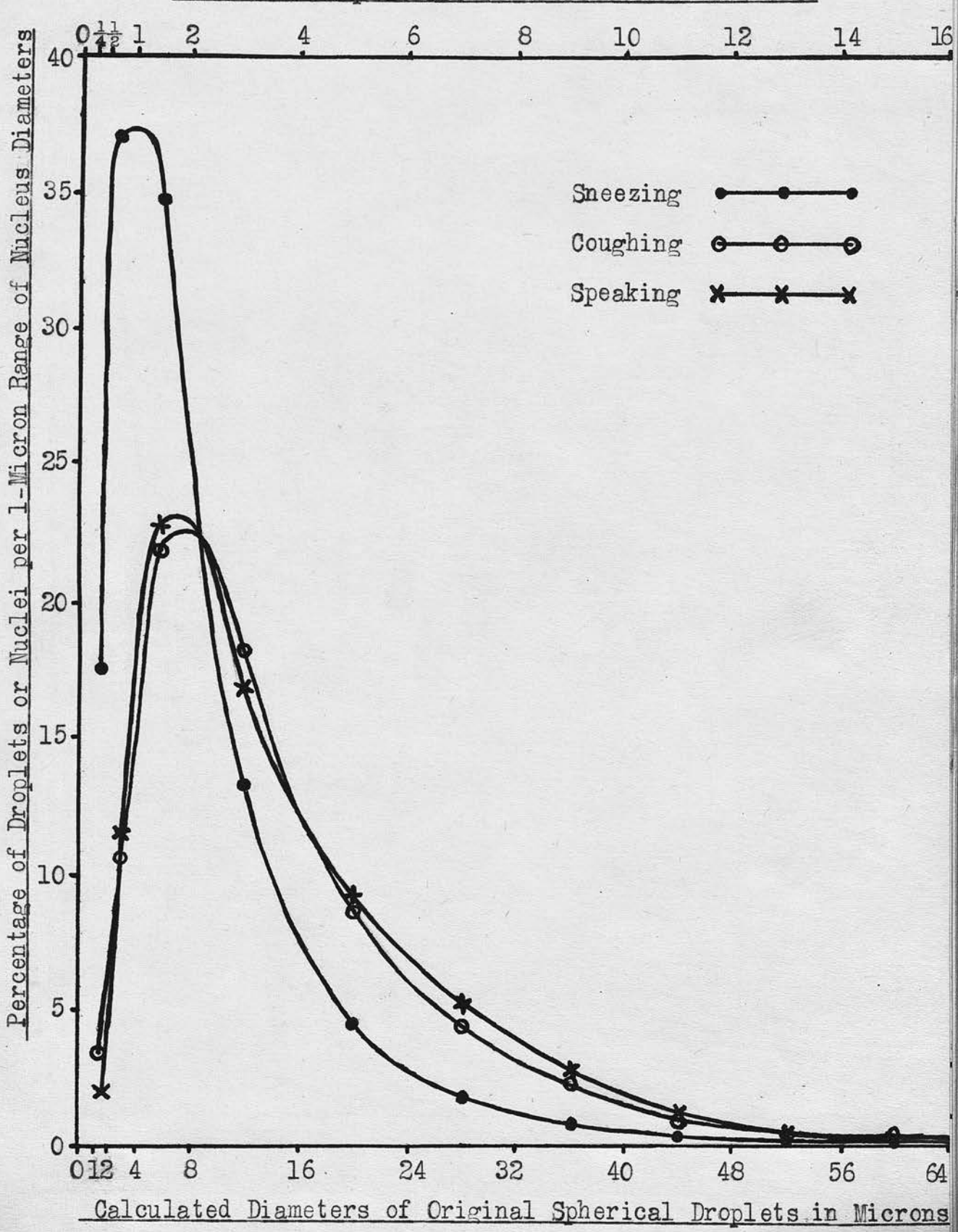
Graph 1 :- Size Distribution of Droplets Large Enough to be Caught by Impingement on Slides Exposed in front of the Mouth Directly to Droplet Spray.

(from results shown in table 14)



Graph 2 :- Size Distribution of Droplet Nuclei and Droplets Small Enough to Become Nuclei.

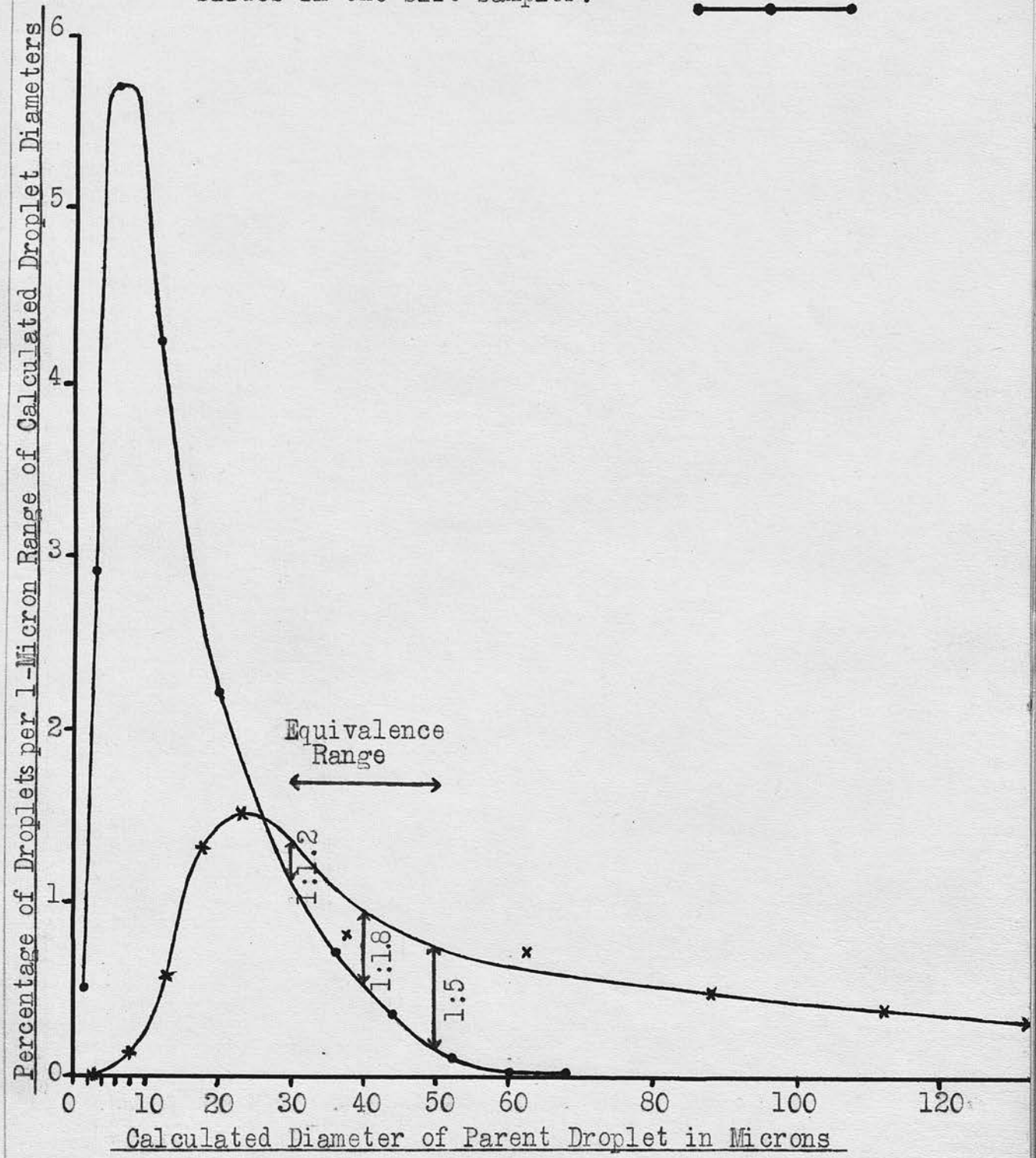
(from results shown in table 14; measurements of droplet nuclei recovered from air on oiled slides in slit sampler)



Graph 3 :- Size Distribution of Speech Droplets as Calculated by the Two Different Methods, from Data in Table 14.

(a) Parent droplet diameters calculated from measurements of droplet marks on slides exposed in front of mouth directly to droplet spray:

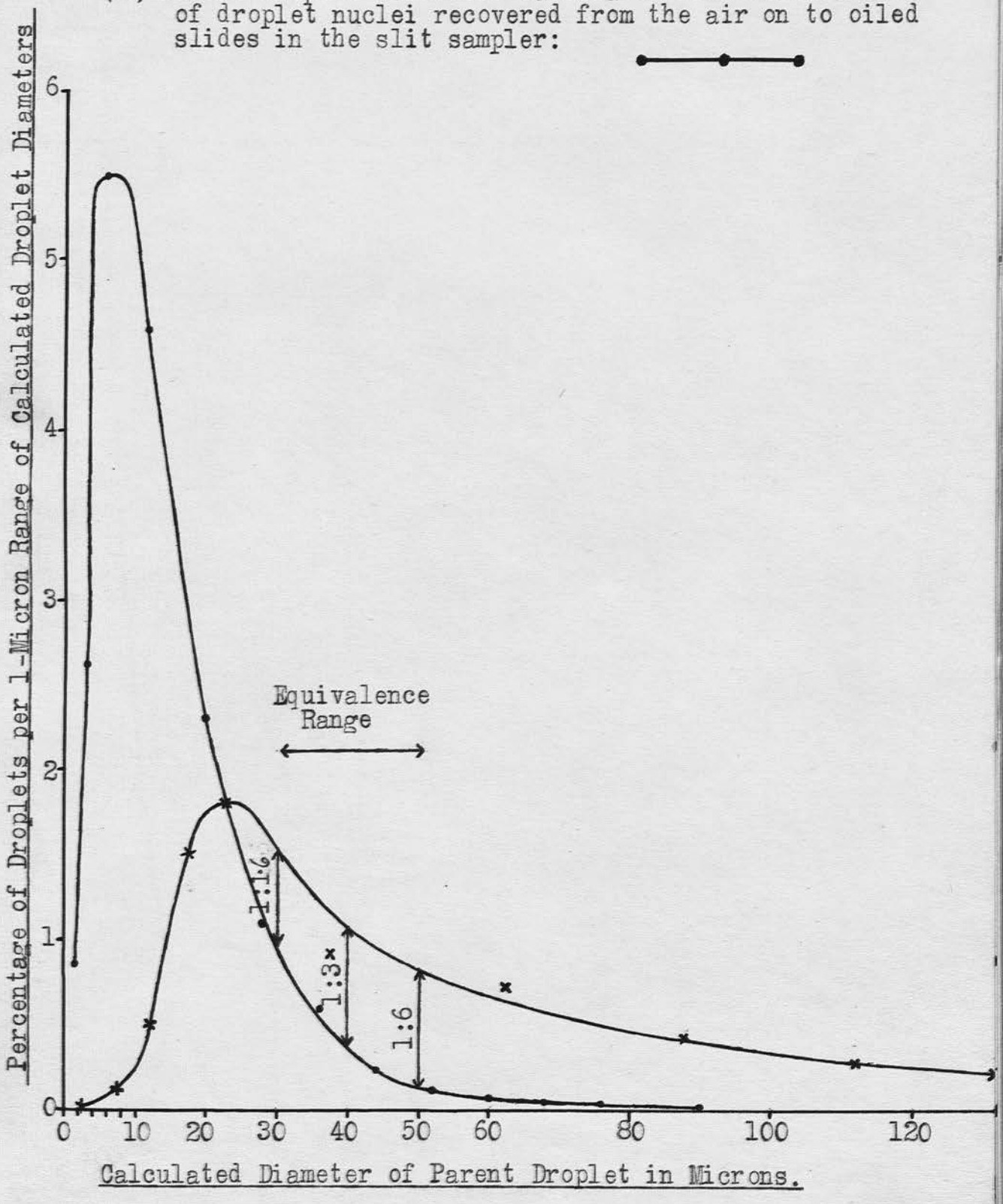
(b) Parent droplet diameters calculated from measurements of droplet nuclei recovered from the air on to oiled slides in the slit sampler:



Graph 4 :- Size Distribution of Cough Droplets as Calculated by the Two Different Methods, from Data in Table 14.

(a) Parent droplet diameters calculated from measurements of droplet marks on slides exposed in front of mouth directly to droplet spray:

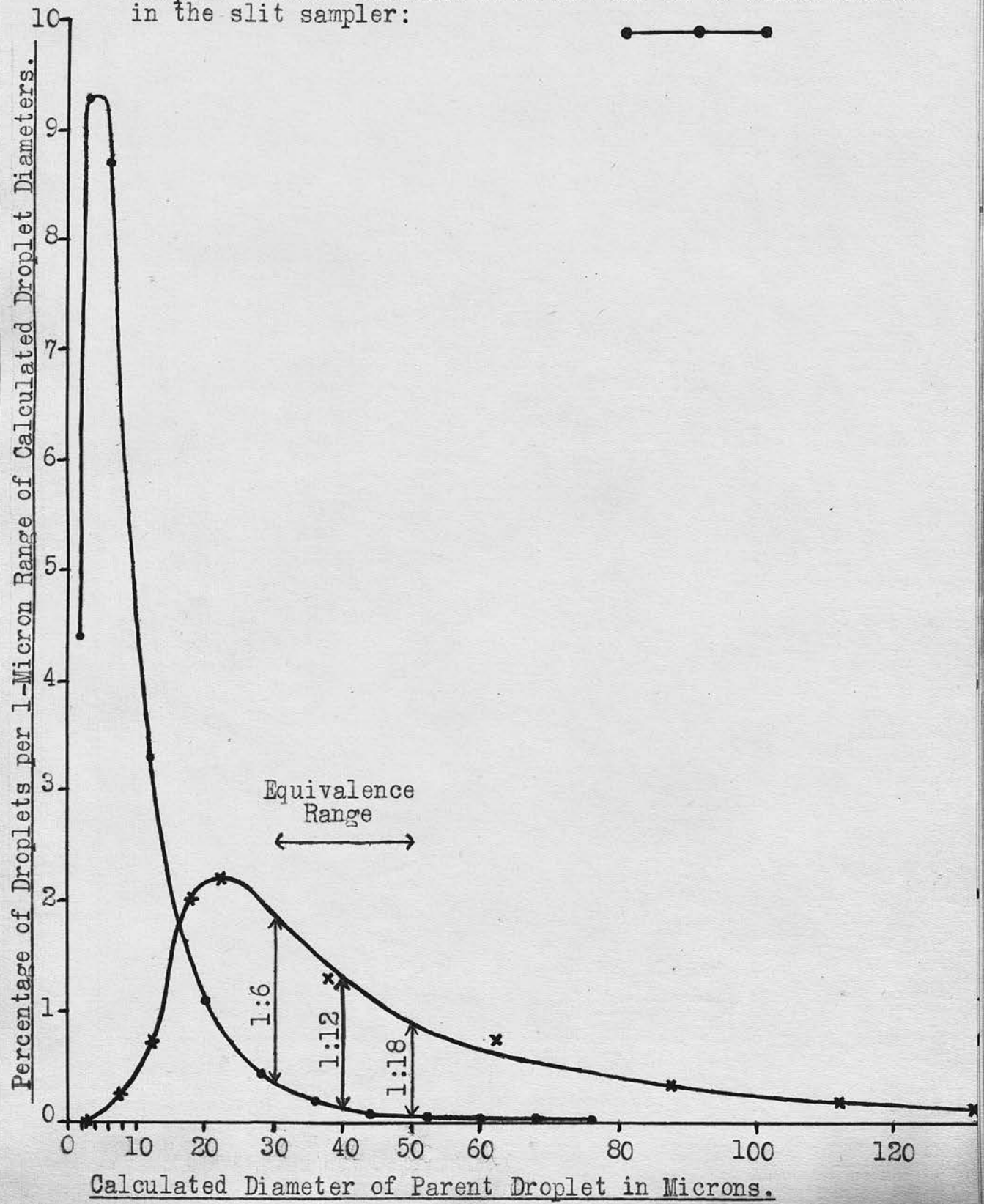
(b) Parent droplet diameters calculated from measurements of droplet nuclei recovered from the air on to oiled slides in the slit sampler:



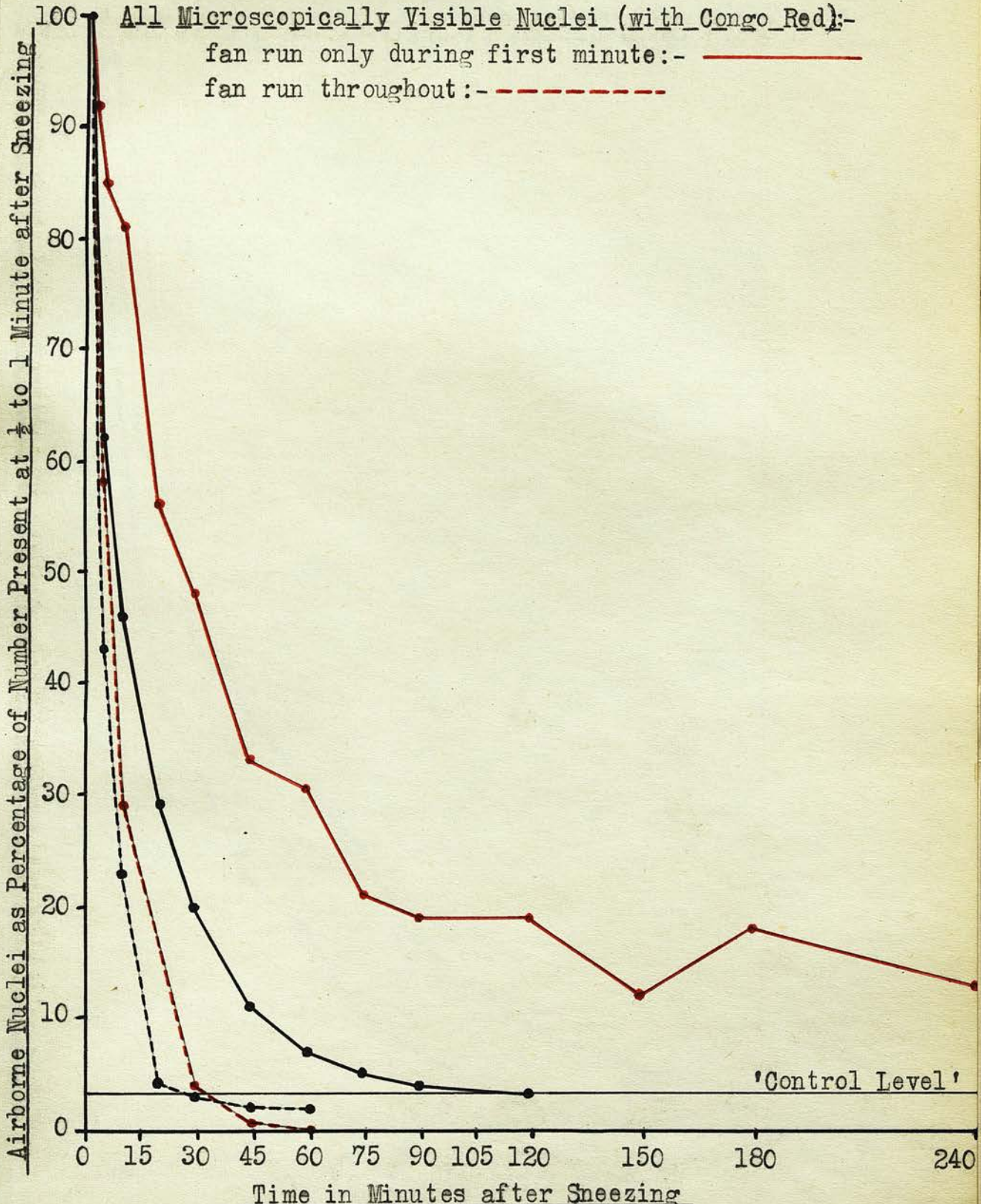
Graph 5 :- Size Distribution of Sneeze Droplets as Calculated by the Two Different Methods from Data in Table 14.

(a) Parent droplet diameters calculated from measurements of droplet marks on slides exposed in front of the mouth directly to droplet spray:

(b) Parent droplet diameters calculated from measurements of droplet nuclei recovered from the air on to oiled slides in the slit sampler:



Graph 6:- Disappearance from Air of 70 Cu.Ft. Chamber of Droplet Nuclei Produced by Sneezing: Curves Constructed from Average Figures given in Tables 24 & 38.



'Control Level' of air infection before sneezing is shown for bacteria-carrying nuclei.

Graph 7:- Disappearance from Air of 1700 Cu.Ft. Chamber of Droplet Nuclei Produced by Sneezing: Curves Constructed from Average Figures given in Tables 22 and 29.

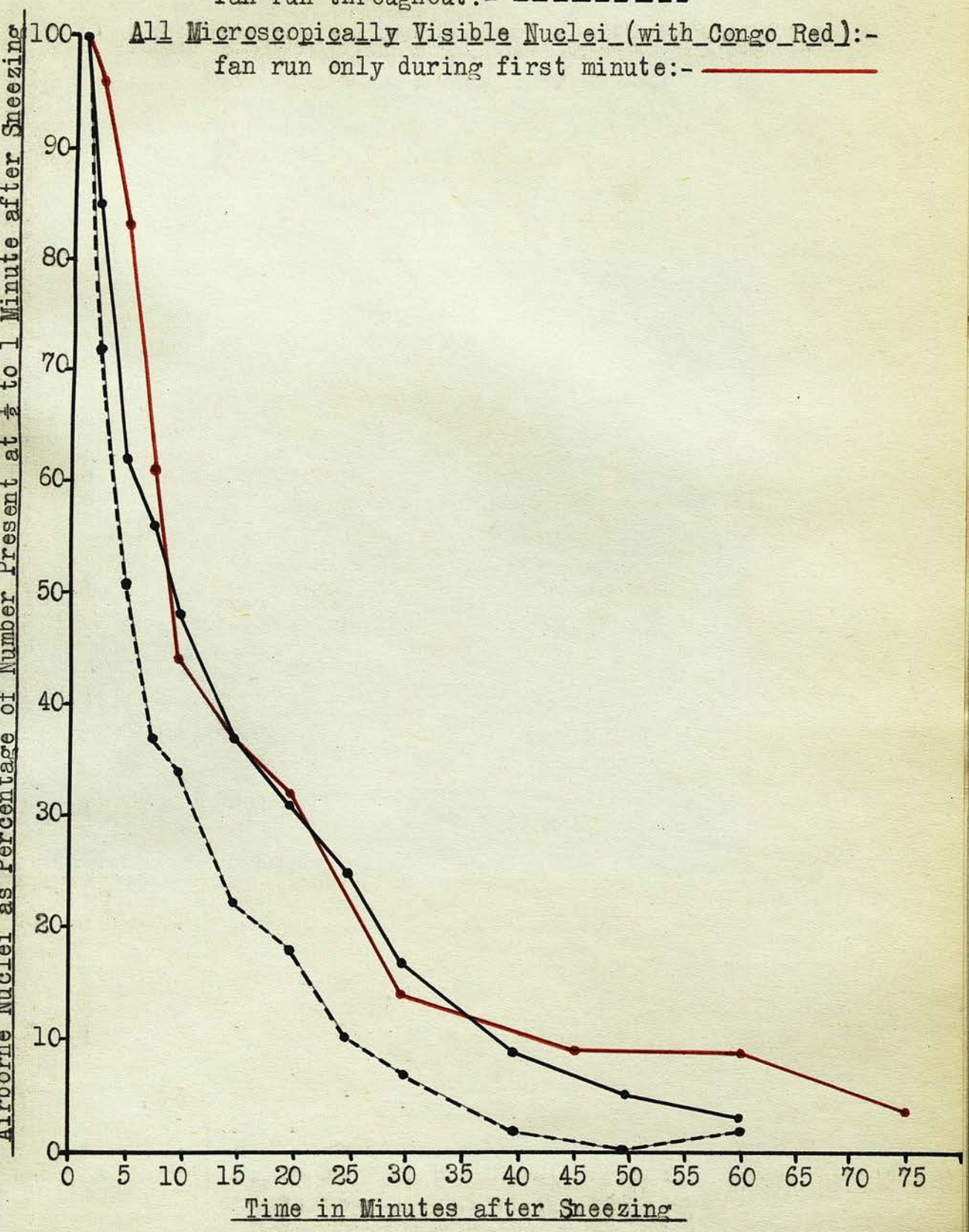


Figure 1:- Microphotograph (x 100) of Congo Red-Containing Sneeze Droplet Marks.

0.1 grams of Congo Red was taken into the mouth and a 'natural sneeze' delivered towards a glass slide held at 6 inches in front of the mouth. After drying, the slide was examined and photographed with the low power of the microscope. The field shows droplet marks ranging in diameter from 20 to 500 microns.

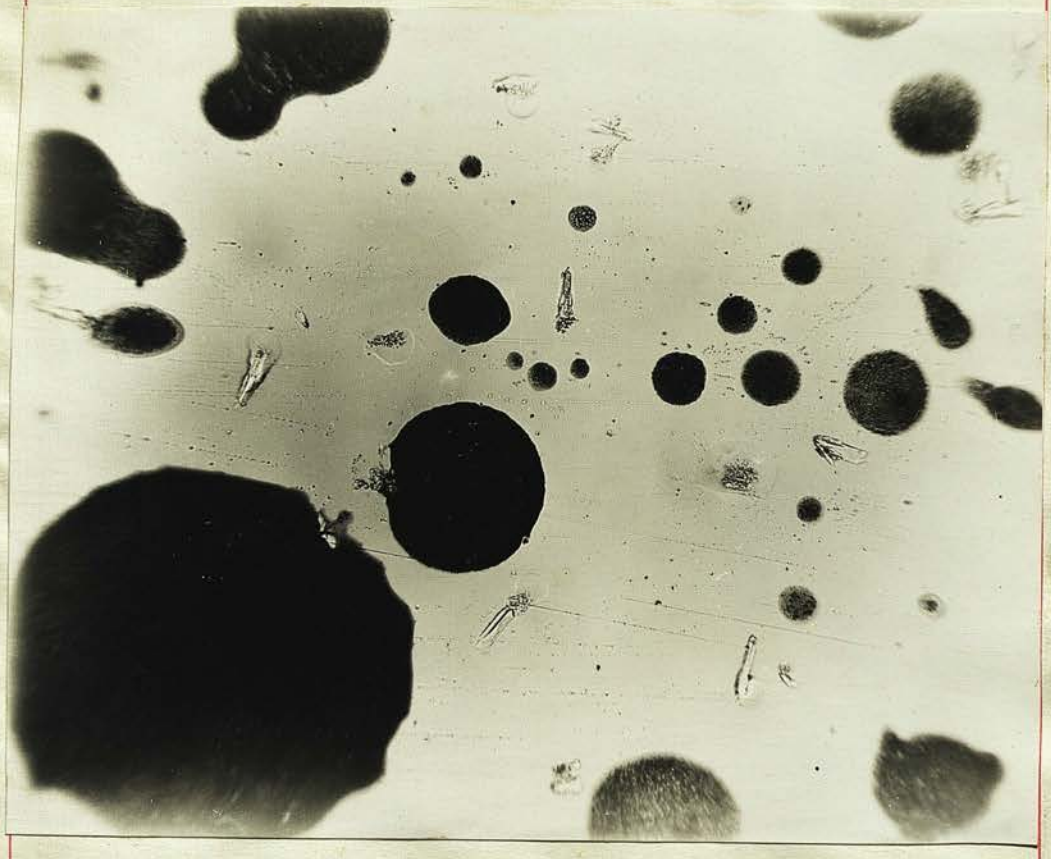


Figure 2:- Microphotograph (x 1000) of a Gram-stained Sneeze Droplet Mark Containing Commensal Bacteria and an Epithelial Cell.

A 'natural sneeze' was delivered towards a glass slide held at 6 inches in front of the mouth. After drying, the slide was fixed by heat, stained by Gram's method and photographed with the oil-immersion objective of the microscope. The field is largely occupied by a single droplet mark with a diameter approaching 100 microns.

Figure 3:- Showing how the dust-line is scanned from side to side in transverse bands as it is passed under the micrometer scale.

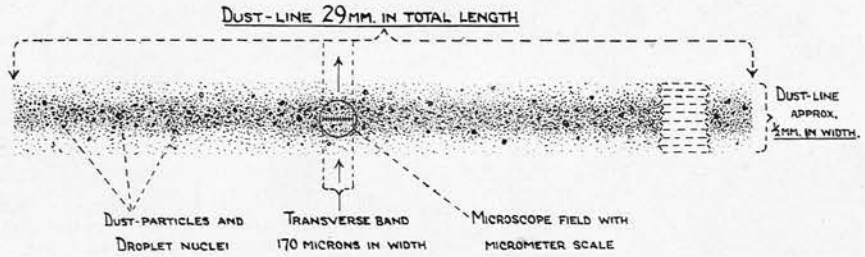
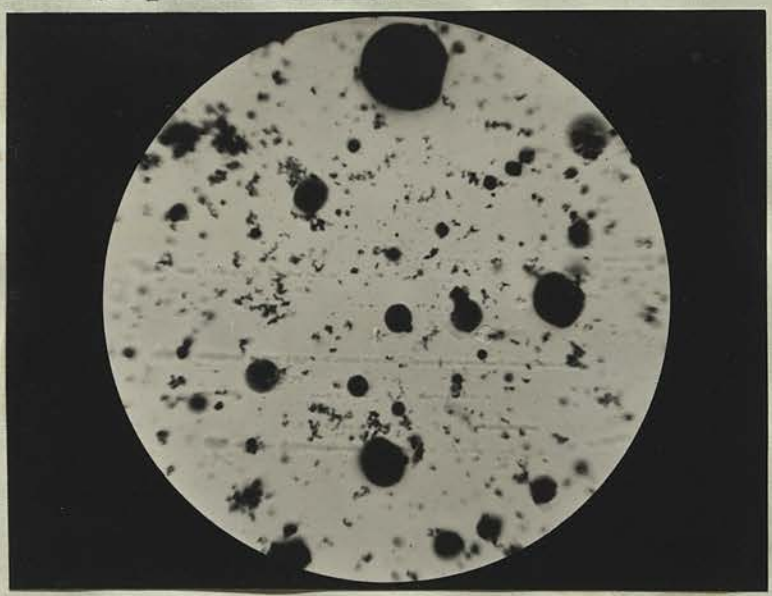


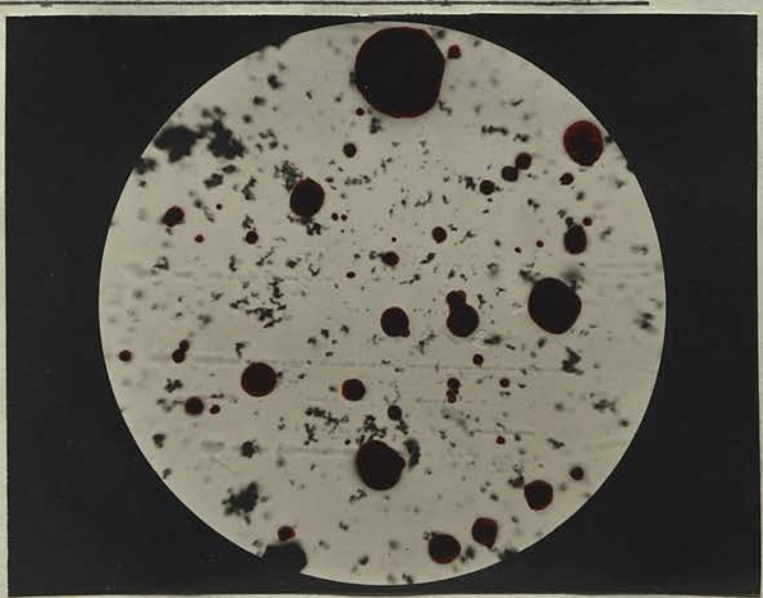
Figure 4:- Microphotograph (x 1000) of Congo Red-Containing Sneeze Droplet Nuclei and Dust Particles from the Air.

0.1 grams of Congo Red was taken into the mouth and a 'natural sneeze' was then delivered in a small room. At one minute after sneezing, air from the room was sampled on an oiled slide in the slit sampler. The slide was examined and photographed with the oil-immersion objective of the microscope. The field shows about fifty droplet nuclei which range in diameter from $\frac{1}{4}$ to 12 microns and are mostly spherical, and also numerous irregularly-shaped dust particles of small size.

(a) Photograph Untouched:-



(b) Droplet Nuclei Touched Up with Red:-



Figures 5 & 6:-Microphotographs (x 1000) of Congo Red-Containing Sneeze Droplet Nuclei on an Oiled Slide Exposed in Slit Sampler.

The two photographs were taken of the same field at different focal depths, using an oil-immersion objective and micrometer eyepiece: they illustrate (a) the three-dimensional solidity of the nuclei, and (b) how the nuclei are measured with the eyepiece micrometer scale (division: 1.7 microns).

Fig.5:- Dust particles, scratches on surface of slide and margins of small nuclei are in focus, but margins of the two largest nuclei are out of focus and blurred.

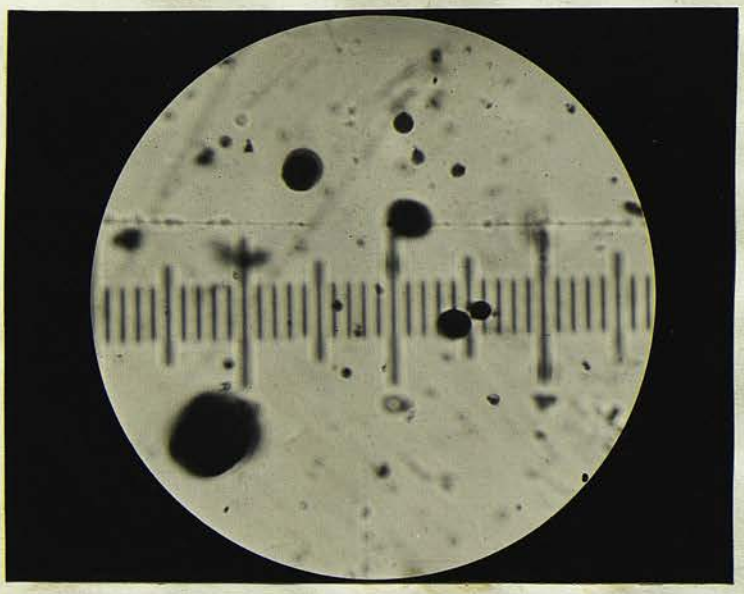


Fig.6:- Dust particles, scratches on surface of slide and margins of small nuclei are out of focus and blurred, while margins of the two large nuclei are sharply focussed.

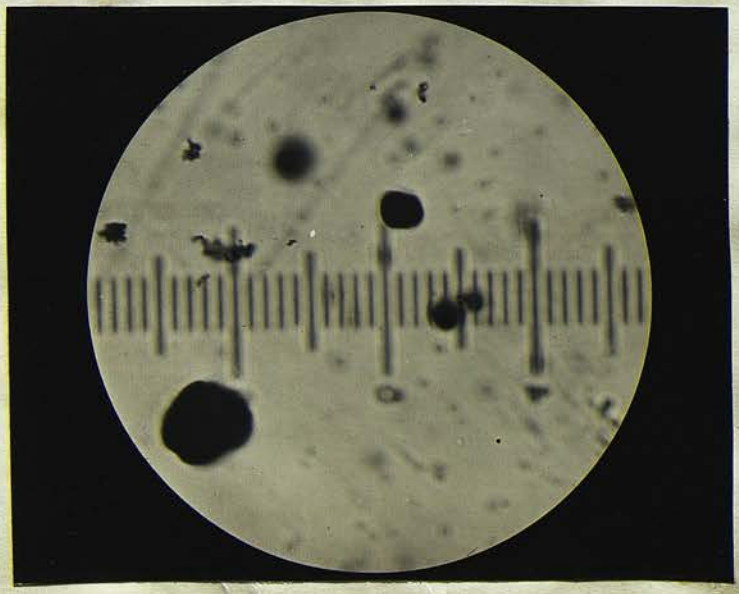


Figure 7:- Drawing of Droplet Nuclei and Dust Particles Recovered from the Air on to Oiled Slides Exposed in the Slit Sampler.

Magnification: x 1000

The droplet nuclei are colored red because of their content of Congo Red, this dye having been taken into the mouth prior to sneezing.

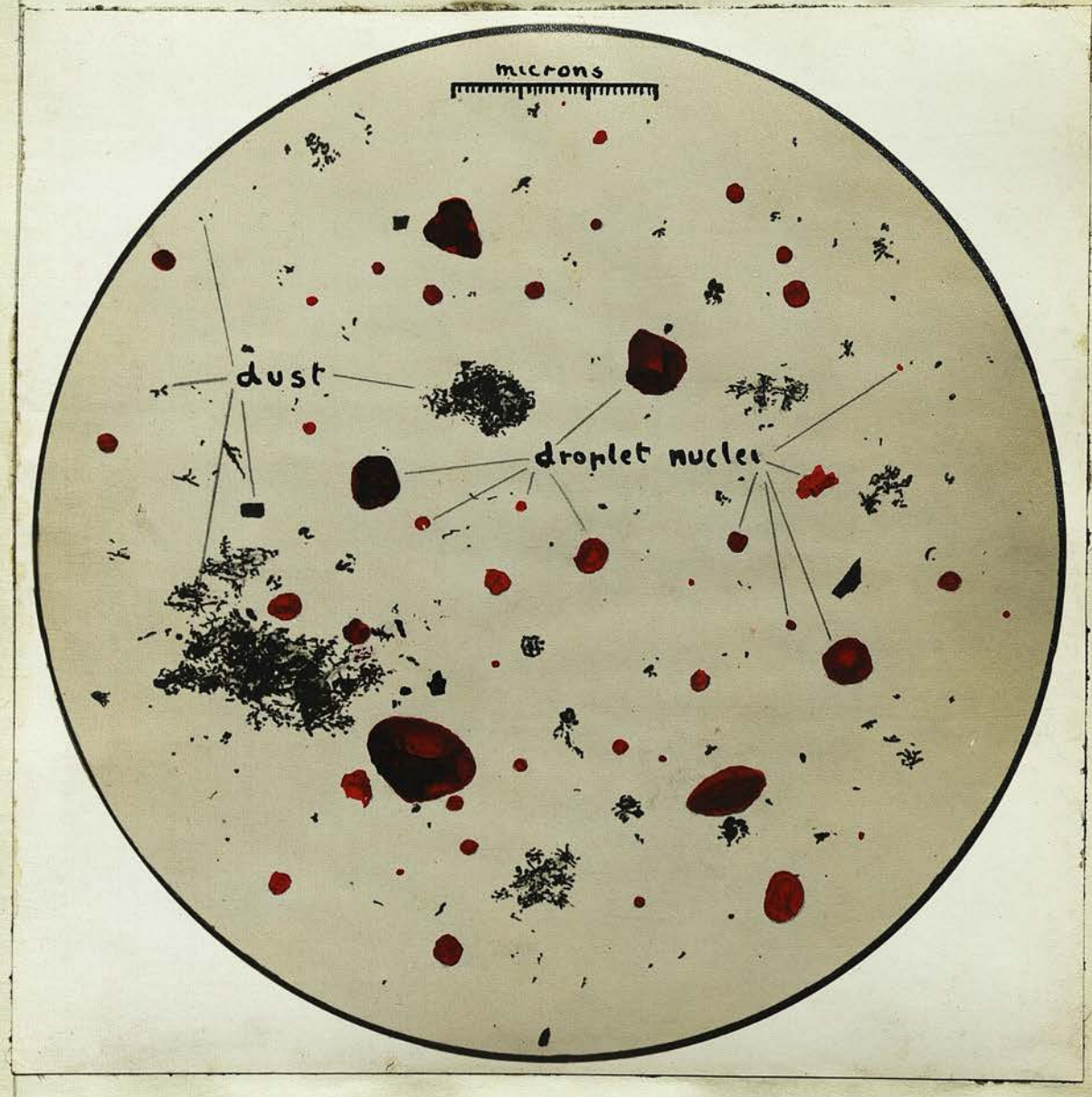


Figure 8:- Drawing of Droplet Nuclei and Dust Particles Recovered from the Air on to Oiled Slides Exposed in the Slit Sampler.

Magnification: x 1000.

The droplet nuclei are colored pink because of their content of Eosin, this dye having been taken into the mouth prior to sneezing.

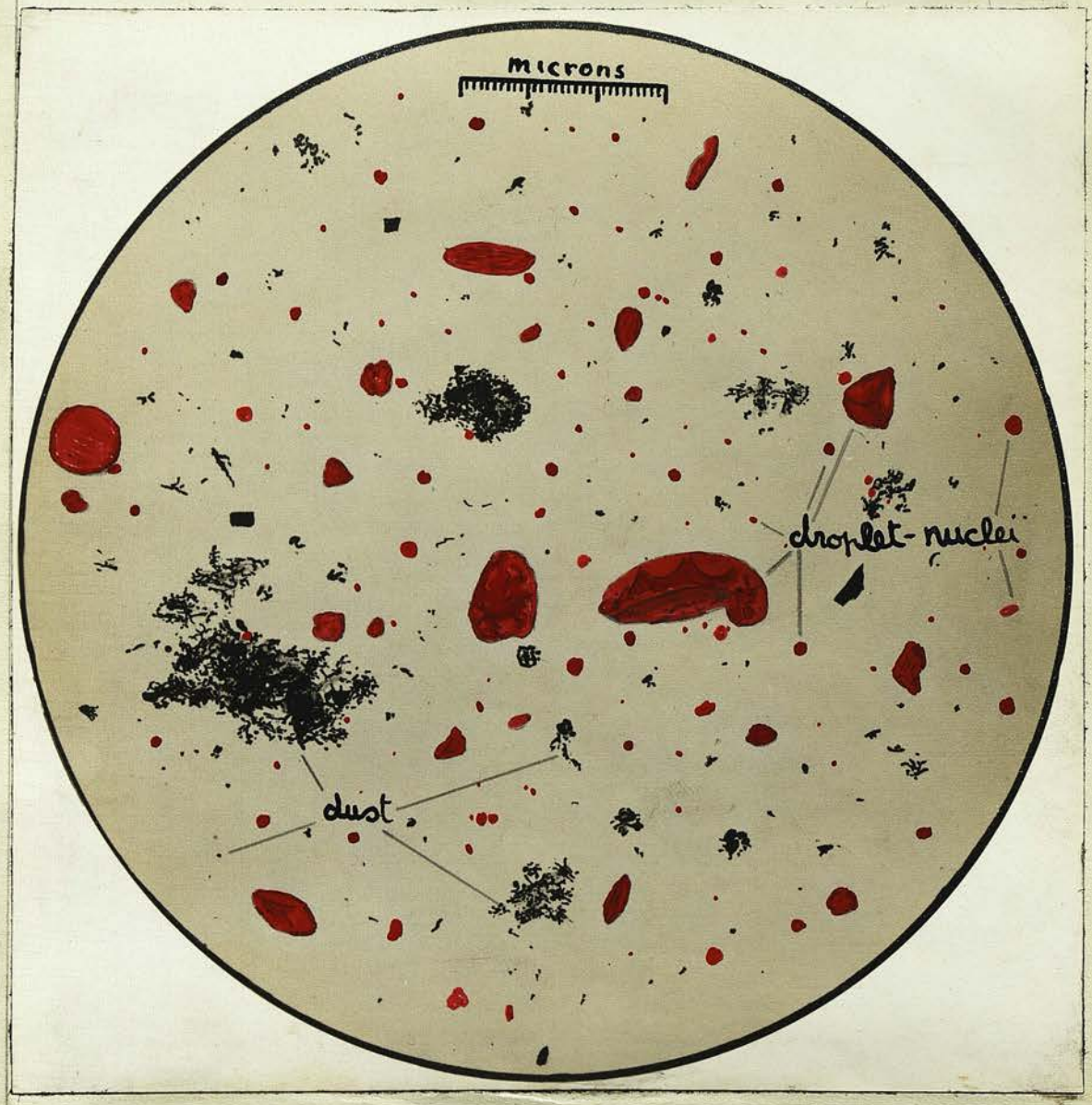


Figure 9:- Drawing of Droplet Nuclei and Dust Particles Recovered from the Air on to Oiled Slides Exposed in the Slit Sampler.

Magnification: x 1000

The droplet nuclei are colored black because of their content of India Ink, this pigment having been taken into the mouth prior to sneezing.

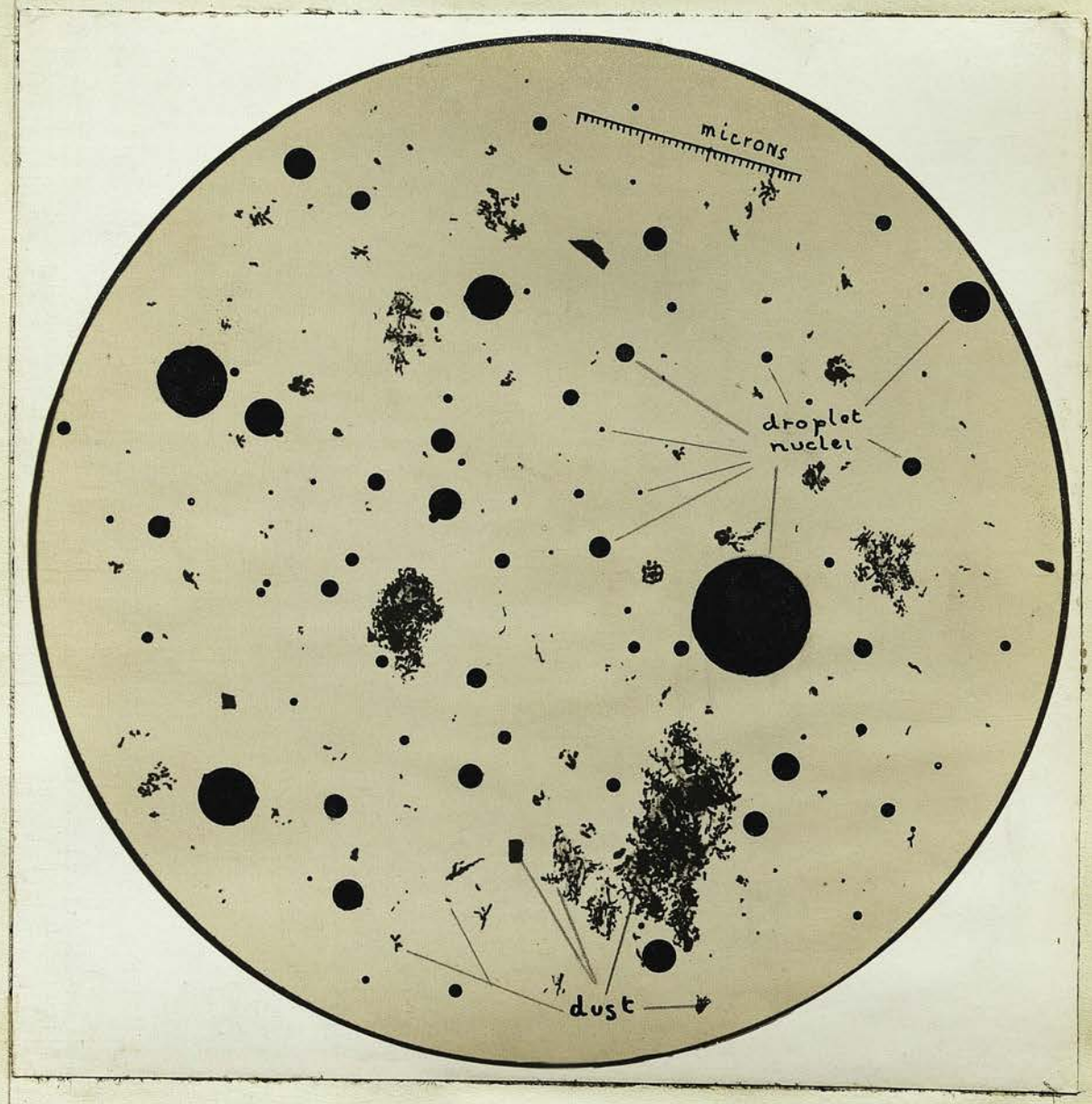


Figure 10;- Photograph of the 100 Cu.Ft. Test Chamber Showing Door, Slit Sampler and Glass Tube Joining Chamber Vent to Sampler Intake.

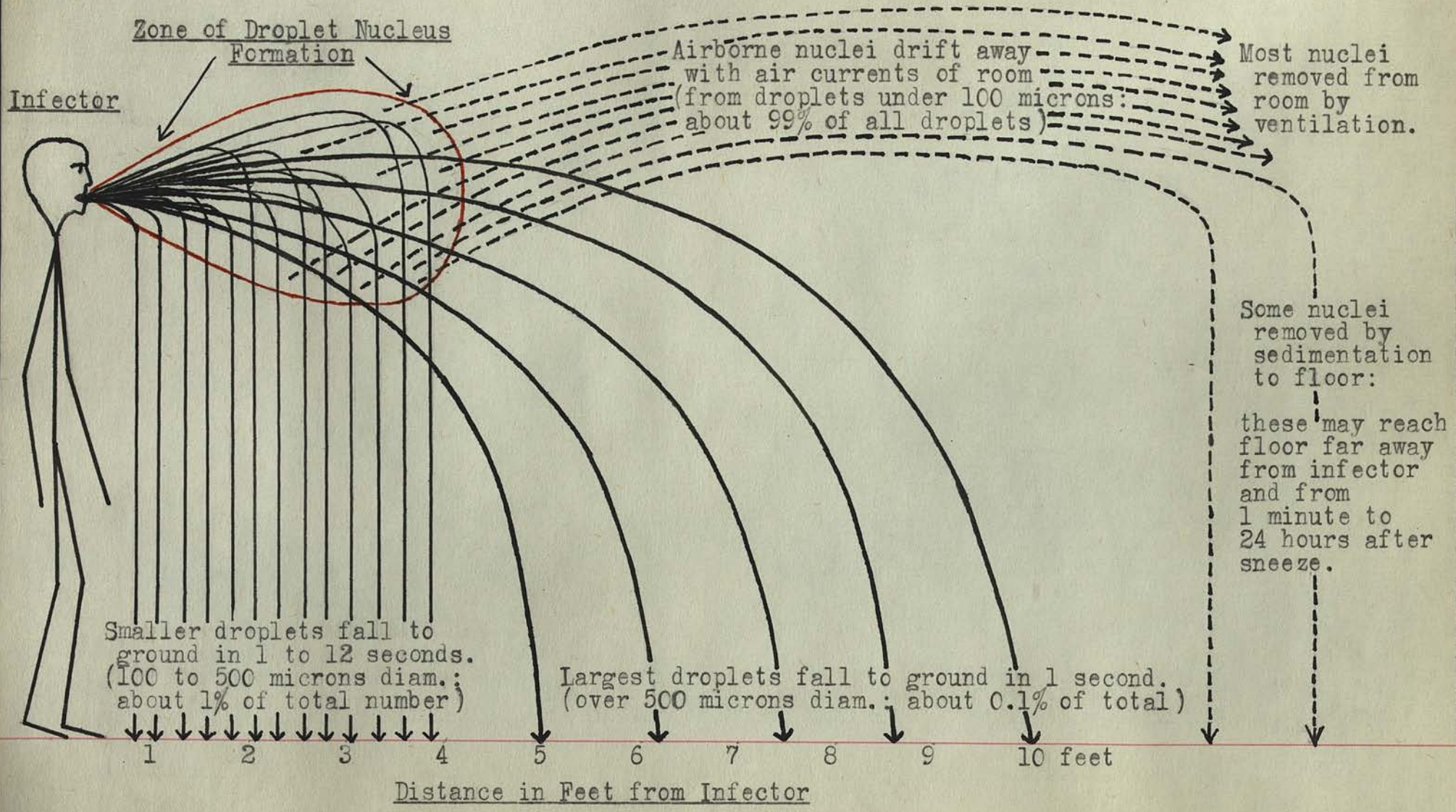
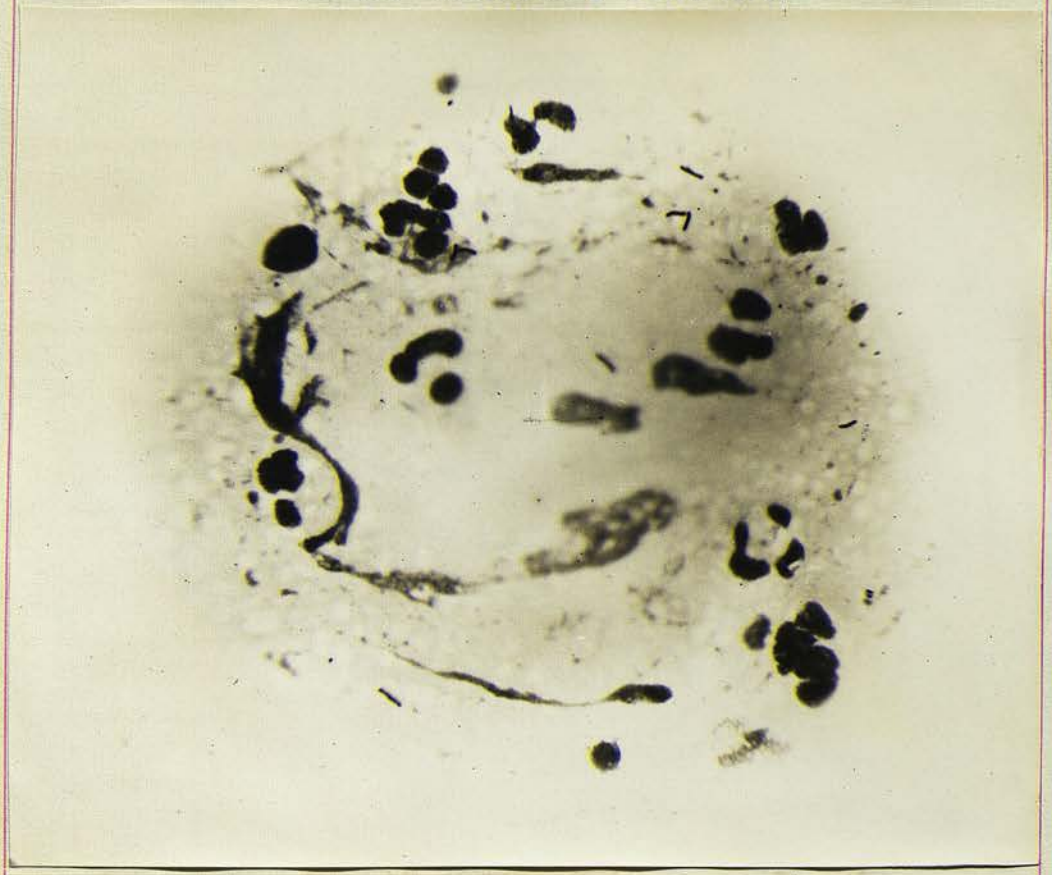
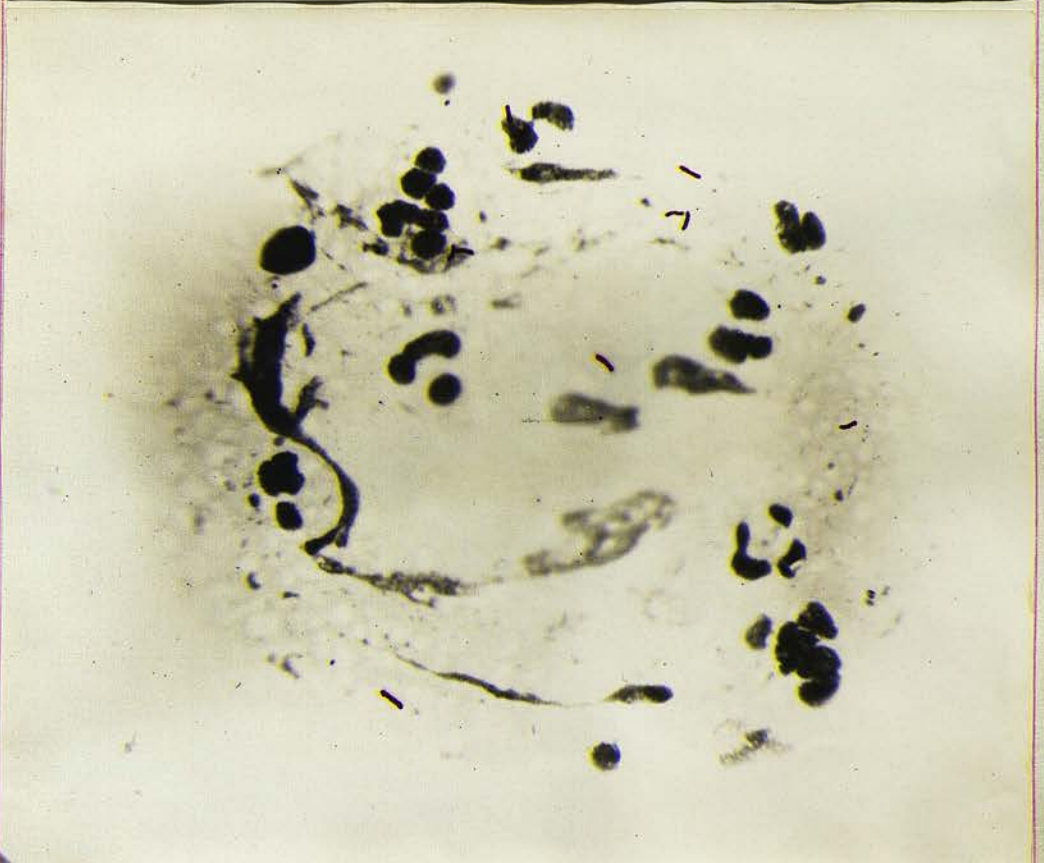


Figure 11:- Diagram of the Droplet-Spray Emission by a Sneeze as Recorded in Sections 12 & 13.

Figure 12:- Microphotograph (x 1000) of a Cough Droplet Containing Tubercle Bacilli.

The slide was exposed during coughing in front of the mouth of a patient with pulmonary tuberculosis; it was stained by the Ziehl-Neelsen method. The droplet mark contains polymorph leucocytes and nine tubercle bacilli. The upper photograph is untouched; in the lower, the tubercle bacilli are touched up with red.





Section 35: Comparison of the sanitary significance of the different expiratory activities in terms of the number of droplets expelled by each.

Tables 1-6 show the droplet counts obtained by the different methods of collection and observation. The calculations described in Section 30 showed that only one method was efficient in revealing a major proportion of the droplets; this was the method which was developed and used for the first time in the present investigation and which involved the microscopical observation of dye-containing droplet nuclei collected from the air on to oiled slides by the slit sampler (Section 18). The droplet counts obtained by this method (Table 4) were much higher than the counts obtained by the other methods and they will be accepted in the present discussion as approximating to the true counts.

The droplet counts obtained in the present study by those counting methods which had been used previously by other investigators, were of the same order of magnitude as the counts obtained by these other investigators. For instance, the method of counting colonies on blood agar used in an air sampler for collection of droplet nuclei (Section 17) yielded, in the present investigation, an average count of 40,000 bacteria-carrying droplets per 'natural sneeze', in the hands of Bourdillon, Lidwell and Lovelock (1942) about 100,000 droplets per sneeze and in the hands of Wells (1935) about 20,000 droplets per sneeze.

The/

The results obtained for normal breathing require special comment. Conflicting opinions have been expressed in the past about the possibility of droplets being expelled in normal respiration. Most authors have concluded that normally expired breath is free from bacteria. However, it it difficult to obtain a sure solution to the problem. It can be shown readily and with certainty that large numbers of infected droplets are not emitted in normal breathing (see Table 1). It is also possible to show that mouth breathing does not expel even a small number of droplets containing salivary streptococci (see Table 1). It is difficult, however, to prove that nose breathing does not expel a small number of droplets, because the air is always infected to some extent with dust-borne bacteria which include species similar to the commensal bacteria of the nose. Thus, with regard to nose breathing, it was necessary to rely on experiments made with an indicator organism, B.prodigiosus, artificially inoculated into the nasal passages. These experiments showed beyond doubt that a few droplets were usually expelled from the nose in 5 minutes of normal nose breathing (Tables 5 and 6). It is quite possible, of course, that these droplets were expelled only by the occasional heavier expiration which can hardly be avoided in the course of breathing; strong expiratory 'sniffs' did, in fact, expel fairly large numbers of droplets (Tables 1 and 3). It/

It must be accepted as probable that Staph.aureus carried in the anterior nares may be expelled in very small numbers during breathing.

Table 7 shows the averages (arithmetic means) of the droplet counts obtained by the different methods. The significance of these average values is limited because of the very large variation between counts for separate expiratory acts of the same kind. The number of droplets expelled was found to vary enormously according to the violence of the expiratory act and according to the wetness of the lips and front parts of the mouth. Thus, droplet counts varied between 65,000 and 16,000,000 for a sneeze, between 0 and 50,000 for a cough and between 0 and 770 for speaking 100 words (Table 4).

The average rate of droplet expulsion was approximately 1000,000 droplets per sneeze, 1000 droplets per cough, 1 droplet per spoken word, 100 droplets per strong nasal expiration, 1 droplet per minute of laughing and 1 droplet per 3 minutes of nose breathing. Comparison of the counts obtained for the larger droplets (Tables 1 and 2) with the counts obtained for the droplet nuclei (Tables 3 and 4) showed that the majority of the droplets expelled by all the different expiratory activities were small enough to remain airborne as droplet nuclei.

In order to assess the danger of droplet spray infection/

infection from each of the different expiratory activities, it is necessary to consider not only the numbers of droplets expelled by each activity, but also the frequency of occurrence of each activity among healthy and ill persons. No figures obtained by mass observation have yet been published for the normal frequency rates of the different expiratory activities. However, it is possible from general experience to estimate roughly the probable limits of this frequency. A healthy person having three or four colds a year probably gives between 10 and 1000 sneezes a year, and so expels between 10,000,000 and 1000,000,000 droplets per year by sneezing. A healthy person may give from 1 to 10 coughs a day, and a patient with respiratory tract disease up to 100 or 1000 coughs a day; thus, by coughing, the healthy person may expel between 400,000 and 4000,000 droplets per year, and the patient between 100,000 and 1000,000 droplets per day of his illness. A normal person probably speaks from 1000 to 10,000 words a day, and so expels between 400,000 and 4000,000 droplets per year. By normal nose breathing a person may expel about 200,000 droplets per year. Laughing is less frequent than speaking and relatively much less productive of droplets; for this reason it may be regarded as unimportant.

On the basis of the above figures, sneezing appears to be by far the most important producer of droplet spray. One sneeze expels about as many droplets/

droplets as are expelled by a person's speaking during one year, by a person's breathing during 5 years, or by a healthy person coughing during one year at the rate of 1 to 10 coughs per day. In fact if a person gives even so few as 10 sneezes in a year, his output of droplets by sneezing will greatly exceed his output of droplets by all other causes, coughing, speaking, breathing and laughing, taken together. Furthermore, the huge droplet output of each sneeze is concentrated in time and space, so ensuring a maximum infecting dose to the persons exposed. The droplet output of speaking and breathing is distributed fairly evenly throughout the year and throughout the many different rooms and other premises occupied by the infector; the resulting air infection is necessarily very dilute.

Apart from the frequent coughing of patients with respiratory tract disease and the frequent sneezing of hay fever patients and snuff adicts, the expiratory activities mentioned above are as much exercised by normal persons, who have 3 to 4 colds a year, as by patients with respiratory tract disease other than the common cold. Thus, with regard to the frequency and amount of droplet spray emission, droplet spray is just as likely to disseminate pathogenic organisms from healthy carriers as from patients with clinically apparent respiratory infection. The 'carrier' of such organisms as Strept.pyogenes, C.diphtheriae, Pneumococcus,/

Pneumococcus and Meningococcus, will become especially dangerous as a source of droplet spray infection when he contracts a cold and becomes liable to frequent sneezing. The sneezes of a person with a cold are probably to be feared more because his droplets may convey pathogenic bacteria which he has been 'carrying' in his throat or nose, than because they may convey the common cold virus.

Section 36: Site of origin of the respiratory tract secretion droplets.

It has been accepted generally and is confirmed by the present observations that the great majority of sneeze droplets, cough droplets and speech droplets originate from the secretion of the anterior region of the mouth. Commonly, pathogenic organisms may be present in the throat or in the nose, and yet be absent from the saliva of the anterior mouth. For this reason it is important to know whether or not a small proportion of the expelled droplets may originate from the throat or nose.

By the photographic method, Bourdillon and Lidwell (1941) and Jennison (1942) showed that in sneezing some droplets are discharged from the nose. In the present investigation, tests of sneezing with the mouth masked (Table 1) and tests of sneezing with B.prodigiosus inoculated in the nose (Tables 5 and 6) showed that from 0 to 5600 droplets were discharged from the nose during a single 'natural sneeze'. On average, approximately 100 droplets per sneeze were expelled from the nose; thus, droplets originating from the nasal passages comprised about 0.01% of the total number of droplets expelled in sneezing. A large proportion of the nasal droplets were small enough to remain airborne as droplet nuclei (Table 6).

Tests with B.prodigiosus inoculated on the tonsils, soft palate and back of tongue (Tables 5 and 6) showed that/

that from 0 to 2300 droplets originating from the throat were emitted during a single 'natural sneeze'. On average, approximately 200 droplets per sneeze were expelled directly from the throat; thus, the throat droplets comprised about 0.02% of all the droplets expelled in sneezing. A considerable proportion of the throat droplets expelled in sneezing were small enough to remain airborne as droplet nuclei (Table 6).

It is thus apparent that in sneezing more than 99.9% of the droplets originate from the saliva of the anterior mouth. If pathogenic organisms are confined to the nose or throat, only a very few of the expelled droplets can be infected.

Tests with B.prodigiosus inoculated in the throat as an indicator of throat origin showed that a cough made with the mouth kept well open, a 'throat-only cough', discharged a few droplets originating from the throat. Between 0 and 279, on average 31, throat droplets were expelled per cough (Table 5). Some of the throat droplets were small enough to remain airborne as droplet nuclei (Table 6). The droplets of throat origin appeared to comprise a major proportion of the total number of droplets discharged by a 'throat-only cough' (compare figures in Table 1 with those in Table 5). It is concluded that only a very few droplets of tonsillar and pharyngeal secretion are expelled by coughing.

A/

A few droplets originating from the throat were found to be expelled by laughing and by speaking loudly words containing the sound, "K", (Tables 5 and 6).

Because only a very small proportion of secretion droplets originate from the throat and nose, droplet spray will be a great danger of infection only if the anterior mouth secretion contains a high concentration of pathogenic bacteria.

Section 37: Size of secretion droplets and nuclei.

The original diameter measurements of 25,551 dye-containing droplet nuclei and the calculated diameters of their parent droplets before evaporation, are shown in Tables 11, 12, 14 and 47, and in Graphs 2, 3, 4 and 5. The original diameter measurements of 15,000 dye-containing droplet deposit marks and the calculated diameters of their parent droplets in the spherical shape, are shown in Tables 9, 10 and 14, and in Graphs 1, 3, 4 and 5. The calculated composite size-distributions for unevaporated spherical parent droplets of all sizes, are shown in Tables 15, 16 and 17.

The droplets at the moment of emission from the mouth varied from about 1 to 2000 microns in diameter. The great majority, 60-85%, were between 2 and 25 microns in diameter. There was not a great deal of difference between sneeze droplets, cough droplets and speech droplets; the sneeze droplets were relatively more numerous in the smaller size groups. The commonest diameters among the sneeze droplets were between 2 and 5 microns, and among the cough droplets and speech droplets between 5 and 10 microns (Graph 2). It was calculated that about 99% of sneeze droplets, 92% of cough droplets and 87% of speech droplets were smaller than 100 microns and thus were small enough to remain airborne as droplet nuclei (Table 15).

The dye-containing droplet nuclei must have been

from/

from 25% to 50% larger in diameter than natural dye-free nuclei because of their 1% to 2% content of Congo red. The dye-containing nuclei found in the air varied from about ¼ to 42 microns in diameter. The great majority of the nuclei were between ½ and 6 microns in diameter. The commonest diameters among sneeze nuclei were about 1 micron and the commonest diameters among cough nuclei and speech nuclei were about 2 microns. Relatively few of the airborne droplet nuclei, between ½% and 2%, were larger than 12 microns in diameter (Table 14). Only 8 out of 25,551 droplet nuclei were larger than 25 microns in diameter (Tables 11 and 47). It is considered therefore that 25 microns represents the upper size limit for droplet nuclei. It follows from this that a droplet of 100 microns diameter (25 x 4) must be about the largest droplet which when emitted at 5 feet above the floor, can remain airborne as a droplet nucleus.

Section 38: Proportion of secretion droplets and nuclei containing commensal and pathogenic organisms.

It is apparent from these measurements of droplets and nuclei just discussed (Section 37) that the great majority of speech droplets, cough droplets and sneeze droplets are small enough to form droplet nuclei which can remain airborne for a considerable time. However, the chances of a droplet containing pathogenic organisms decrease markedly with the droplet size. It is therefore important to know whether or not an appreciable proportion of the droplets small enough to become airborne nuclei are likely to contain pathogenic organisms. Table 18 shows the percentage of droplets likely to be infected in each size group as calculated by assuming a Poisson-type distribution of the viable microbial units among the droplets of each size. The calculations show that a very low proportion of the smaller droplets are likely to be infected when the anterior mouth saliva contains between 1000 and 1000,000 pathogenic bacteria per milliliter (the concentrations of Strept.pyogenes found in most carriers by Hamburger, 1944). On the basis of these figures and of the droplet size-distributions shown in Table 17, it was calculated that of 1000,000 droplets expelled by a sneeze, only from 350 to 16,000 would be infected, and of these infected droplets only from 6 to 5600 would be small enough to remain airborne as droplet nuclei. Of 1000 droplets expelled by a cough, only 2 to 88 would be infected, and of these infected droplets, between/

between 0 and 19 would be small enough to remain airborne as droplet nuclei. Of 1000 droplets emitted in speaking 1000 words, in say 1 hour, only 3 to 130 would be infected, and of these infected droplets between 0 and 30 would be small enough to remain airborne as droplet nuclei.

These calculations that between 0.2% and 13% of cough droplets and speech droplets will be infected, agree well with the direct observations of Hare (1940) of Strept.pyogenes in the cough-spray and speech-spray of throat carriers; Hare found that between 0.3% and 3.5% of the expelled droplets were infected. The present calculation that only between 0.002% and 3% of cough droplets and speech droplets will become infected airborne nuclei, agrees with Hare's failure to find Strept.pyogenes-infected nuclei in the air sprayed by coughing and speaking.

The calculated figures for droplets and nuclei which will contain viable commensal bacteria, agree well with the figures obtained by direct observation in the present investigation. On the basis of the composite size-distribution figures of Table 17 and the infected percentage figures of the first column of Tables 18 (which assume 30,000,000 viable bacterial units per milliliter of saliva), it was calculated that a 'natural sneeze' would produce 65,000 bacteria-carrying droplet nuclei, that a cough would produce 170 bacteria-carrying nuclei, and that speaking 100 words/

words would produce 21 bacteria-carrying nuclei; these figures are of the same order of magnitude as the observed average figures shown in Table 3, namely 39,000 bacteria-carrying nuclei per 'natural sneeze', 8 to 730 bacteria-carrying nuclei per cough, and 13 to 71 bacteria-carrying nuclei for speaking 100 words. This general agreement between the observed and calculated numbers may to some extent be taken as confirming the validity of the size-distributions proposed (Tables 15 and 17) and of the methods of calculation (Sections 22, 25, 26, 27 and 29).

The general conclusion suggested by these calculated infected percentages for droplets and nuclei, is that airborne infection by droplet nuclei must be of infrequent occurrence and of slight degree. It seems that airborne infection by droplet nuclei will be a danger only when, by sneezing, a huge number of nuclei are produced and when there is a high concentration of pathogenic organisms in the anterior mouth saliva to ensure that an appreciable proportion of the nuclei are infected. The maximum extent of airborne infection is probably obtained on those rare occasions when, with the high concentration of 1000,000 pathogenic organisms per milliliter of saliva, a sneeze produces about 5000 infected airborne nuclei which disperse in the course of a minute or so throughout a room of moderate size to give a concentration of about one infected nucleus per cubic foot of air.

Section 39: Sedimentation rate and duration of air carriage of secretion droplets and droplet nuclei.

Sedimentation rates for droplets and nuclei of different size were calculated by a variety of methods from observations made in the present investigation (Sections 31 and 32). Table 53 shows these observed sedimentation rates and also the sedimentation rates calculated from Stokes' law by Wells (1934). The observed rates for each particle size show close agreement with the rates calculated according to Stokes' law. The rates of fall are such that a particle in still air would fall through 1.7 meters to the ground in 1 second if its diameter were 1000 microns or more, in 1-2 seconds if 200 microns, in 6 seconds if 100 microns, in 2 minutes if 20 microns, in 15 minutes if 10 microns, in 1 hour if 5 microns and in 18 hours if 1 micron. However, the air in a room is normally in circulation, moving round the room in one or two minutes. Therefore, these times for falling through 1.7 meters of air would be the actual times taken to reach the ground at 1.7 meters below, only in the case of droplets larger than 100 microns in diameter and having a falling time which is short in relation to the air circulation time. The largest droplet nuclei, 20 to 25 microns in diameter, have a 1.7-meter falling time, about 2 minutes, which is of the same order of magnitude as the usual circulation time of air in a small room. In consequence, droplet nuclei of all sizes will be maintained, by the circulation of the air, evenly/

evenly distributed throughout the room. Droplet nuclei of any one size will reach the floor after quite different times; their rate of deposition on the floor has been calculated by Phelps and Buchbinder (1941) to equal their sedimentation rate divided by the height of the chamber: that is, in each successive second, there will be deposited on the floor a fraction of the remaining airborne nuclei equal to their sedimentation rate in meters per second divided by the height of the chamber in meters. Thus, nuclei of any given size will show a geometric rate of deposition on the floor, and so also of disappearance from the air. The observed disappearance rate of nuclei of all sizes did, in fact, to some extent conform to the geometric pattern (Graphs 6 and 7).

Tables 21-43 show the disappearance of droplet nuclei from the air of a contaminated chamber as observed in individual experiments. Table 44 and Graphs 6 and 7 show the average percentage disappearance rates under different experimental conditions. The disappearance rate of all microscopically visible nuclei was slower than the disappearance rate of nuclei containing viable commensal bacteria; this was to be expected in view of the larger average size of the latter (Table 18; column 1). The difference was marked in experiments in the 70 cu.ft. chamber (Graph 6). In experiments with sneezing and without the fan run throughout, the time for 90% disappearance from the air/

air of the 70 cu.ft. chamber was from 120 to 660 minutes for all microscopically visible nuclei and from 30 to 60 minutes for bacteria-carrying nuclei; the average equivalent ventilation rate during the first hour was 1.2 overturns per hour for all microscopically visible nuclei (Table 38) and 2.7 overturns per hour for bacteria-carrying nuclei (Table 24). In the 1700 cu.ft. chamber, the disappearance of all microscopically visible nuclei was not so slow, not much slower than the disappearance of bacteria-carrying nuclei (Graph 7); in sneeze experiments without the fan run throughout, the average equivalent ventilation rate was 3.4 overturns per hour for all microscopically visible nuclei and 3.5 overturns per hour for bacteria-carrying nuclei. The 1700 cu.ft. chamber was not so well sealed against ventilation as the 70 cu.ft. chamber, so that the faster disappearance rates in the former chamber may in part have been due to ventilation (possibly approaching 1 overturn per hour).

Continuous rapid circulation of the air by the fan was found to increase the rate of disappearance of the nuclei, particularly in the 70 cu.ft. chamber (Table 44; Graphs 6 and 7). Presumably the great turbulence produced by the fan in the small chamber increased the rate of impingement of the nuclei on the sticky, oiled walls. Continuous running of the fan increased the disappearance rate by 3-fold to 4-fold in the 70 cu.ft. chamber and by nearly 2-fold in the 1700 cu.ft. chamber.

Greatest/

Greatest interest, from the sanitary point of view, attaches to the maximum time for which droplet nuclei containing pathogenic organisms can remain in the air of a room following contamination by speaking, coughing or sneezing. Following sneezing in the unventilated test chambers, the maximum duration of air carriage observed was 30 hours for microscopically visible droplet nuclei about 1 micron in diameter (Table 30), 8 hours for nuclei containing B.mesentericus spores which had been added to the saliva to the extent of about 1000,000,000 per milliliter (Table 54), and 2 hours for nuclei containing viable commensal bacteria which were present in the saliva to the extent of about 30,000,000 per milliliter (Table 23). Almost certainly, viable pathogenic bacteria will never be present in saliva in larger numbers than the viable commensal bacteria (30,000,000 per milliliter), and will usually be present in much smaller numbers (e.g. 1000 to 1000,000 per milliliter as found by Hamburger, 1944, for Strept.pyogenes). This suggests that after an infected person sneezes in an unventilated room, air contamination with nuclei containing pathogenic bacteria will never persist for longer than 2 hours, and will usually last for a much shorter time than 2 hours. In a room ventilated to the usual extent of between 1 and 10 overturns per hour, the duration of air infection will be further shortened.

It seems very unlikely that a sneeze ever produces more/

more than 40,000 nuclei containing pathogenic bacteria, if indeed it ever produces as many (Table 19). In an unventilated room, sedimentation will remove nuclei from the air at the equivalent ventilation rate of about 3 overturns per hour (Table 24); at this rate all infected nuclei would disappear in 5¼ hours. In a room poorly ventilated at the rate of 1 overturn per hour, the total equivalent ventilation rate of 4 overturns per hour would remove all infected nuclei from the air in 4 hours. In a room well ventilated at the rate of 10 overturns per hour, the total equivalent ventilation rate of 13 overturns per hour would remove all infected nuclei from the air in 1¼ hours. These calculated maximum durations of air infection following sneezing, illustrate the influence of ventilation.

From these various observations and calculations it is concluded that the maximum time following spray production for which the air of a room can remain contaminated with droplet nuclei containing pathogenic bacteria, is from 1 to 4 hours. Usually air infection will persist for a much shorter time, perhaps for only a few minutes.

Section 40: Time of evaporation of secretion droplets. Droplet nucleus formation.

The observations described in Section 33 gave the droplet evaporation times recorded in Tables 13, 48, 49, 50, 51 and 52; these are summarised as averages in Table 55. The approximate average observed evaporation time was 1200 seconds for a droplet of 1000 microns in diameter, 300 seconds for a 500-micron droplet, 48 seconds for a 200-micron droplet, 12 seconds for a 100-micron droplet and 3 seconds for a 50-micron droplet. These evaporation times for saliva droplets were observed at about 60% relative humidity and 16°C.. The demonstration by momentary air sampling that droplet nuclei of up to 12 microns in diameter were formed within 1 to 2 seconds after a sneeze (Tables 49 and 50), shows beyond doubt that droplets smaller than about 50 microns in diameter, that is the great majority (75% to 96%) of all droplets, evaporate to form droplet nuclei within 1 to 2 seconds after leaving the mouth.

Wells (1934) by comparing calculated 2-meter falling times (Table 53, last column) with calculated evaporation times (Table 55, column 1), concluded that "somewhere between 0.1 and 0.2 millimeters lies the droplet size which identifies droplets of mouth spray that reach the ground within the life of the droplet as against droplets that evaporate and remain in the air as droplet nuclei". A similar comparison of the observed 1.7-meter falling times (Table 53) with the observed/

observed evaporation times (Table 55), which were obtained in the present investigation, suggests also that the largest droplet capable of becoming a droplet nucleus will be between 200 microns and 50 microns in diameter.

The sedimentation observations described in Section 32 showed that 15 seconds was about the maximum time occupied by an incompletely evaporating droplet in falling through 1.7 meters from mouth to floor. Thus, 15 seconds must be about the maximum time of aerial flight available for droplet nucleus formation, in fact the evaporation time of the largest droplet capable of becoming an airborne nucleus. The latter must have a diameter of about 100 microns, since an evaporation time of nearly 15 seconds ("approximate average": 12 seconds) was calculated for 100-micron droplets from the observed evaporation times of droplets of other sizes (namely, time for 100-micron droplet equals time for X-micron droplet, as in Table 55, multiplied by 100^2 divided by X^2; see Section 32).

With very rare exceptions the largest droplet nuclei found in the air were 25 microns in diameter (Sections 23 and 24; Tables 11 and 47). Calculation of the diameter of the unevaporated parent droplet as 4 times the nucleus diameter gives 100 microns as the diameter of the largest droplet capable of becoming an airborne nucleus.

In/

In these different ways, then, the present experimental observations on secretion droplet evaporation and on droplet nucleus formation directly confirm the hypothesis and the calculations of Wells (1934). The calculated evaporation times accepted by Wells were about one eighth of the evaporation times observed in the present investigation. The falling times accepted by Wells were calculated according to Stokes' law for droplets remaining constant in size (i.e. not evaporating) throughout the fall; these times were much shorter than the observed times of fall of evaporating and shrinking droplets about 100 microns in diameter. As both the observed falling times and the observed evaporation times were longer than the times accepted by Wells, calculation based on these observed times yielded a value, 100 microns, for the diameter of the largest droplet capable of becoming an airborne nucleus, which was not very different from the value calculated by Wells (namely, 145 to 128 microns for 50% to 70% relative humidity).

Section 41: Droplet projection distance. Range of infection by droplet spray.

The observations recorded in Sections 12 and 13 showed that the maximum projection distance of the large secretions droplets was about 10 feet for sneezing, 8 feet for coughing and 5 feet for speaking. The great majority of the droplets were expelled only to about 3 or 4 feet. These "projectile droplets" may thus bespatter and infect the skin and clothing of a person standing within a few feet directly in front of the droplet spray producer.

Airborne, droplet nucleus infection which is received by inhalation, is potentially effective over greater distances than surface infection of skin and clothing by the large projectile droplets. Natural circulation of air within a medium sized room will distributed droplet nuclei to all parts of the room within a few minutes. Air carriage from one room to another in the same building must be considered possible. However, because only a few pathogen-containing nuclei are produced even by sneezing (Table 19), the danger of infection must become very small when these few infected nuclei are diluted in large volumes of air, as they necessarily will be during their distribution to any considerable distance from their source. It seems that the danger of airborne droplet nucleus infection will be great only when the potential recipient is close to the spray producer, particularly when the recipient's head/

head is within the "zone of droplet nucleus formation" which extends forwards from the mouth of the spray producer for about 4 feet (see Figure 11).

Section 42: Conclusions.

Respiratory droplet spray may spread infection in two ways: the small droplets, after becoming nuclei, remaining airborne and travelling far throughout a room or building, may cause infection by inhalation; the large projectile droplets, after an aerial flight of less than a second, may bespatter the skin and clothing of a recipient and so cause surface infection.

Although droplets small enough to become airborne nuclei are produced in large numbers by sneezing and in small numbers by coughing and speaking, only a small proportion of these nuclei are likely to be infected when the saliva contains pathogenic organisms in usual concentrations. Airborne infection by droplet nuclei is likely to occur only when the infector carries the pathogenic organism in very large numbers in the saliva of the front of his mouth, when it is by sneezing that droplet spray is produced and when the recipient is exposed closely to the sneeze, standing within a few feet directly in front of the infector at the time of sneezing. Airborne infection by droplet nuclei is very unlikely if the recipient is not present in the same room as the infector during the time of spray production and does not enter that room within the few minutes following spray production.

Surface infection of the recipient's skin and clothing by large projectile droplets will occur only when the recipient stands within a few feet directly in front of the infector during spray production.

Section 43: Summary.

(1) A variety of experimental methods were employed to investigate those physical characteristics of respiratory droplet spray which relate to its facility for transmitting infection. In particular, the following were studied: the morphology, numbers, sites of origin, sizes, infected proportions, projection distances, air carriage times, sedimentation rates and evaporation times of the droplets and droplet nuclei produced by sneezing, coughing, speaking, laughing and breathing.

(2) Evidence was obtained experimentally which confirmed in its main physical aspects the droplet nucleus hypothesis of Wells (1934), but calculations as to the probable numbers of nuclei which will contain pathogenic organisms led to the conclusion that airborne infection by droplet nuclei will be a much lesser danger, more dilute, short-lived and localised, than was envisaged by Wells.

(3) The microscopical appearance of droplets and droplet nuclei was studied, described and recorded by drawings and photomicrographs. Microscopical demonstration of droplet nuclei was achieved for the first time in the present investigation. The nuclei showed depth of focus and, in many cases, irregular shape; this appearance of 'solidity' was evidence that almost all water had been lost by evaporation.

(4)/

(4) Naked-eye observation of droplet spray by dark-ground illumination with sunlight, revealed that very large numbers of droplets were expelled by sneezing and relatively small numbers by coughing and speaking. Coughing was seen to expel droplets only when the mouth was closed by approximation of the lips or teeth at the beginning of each cough. The great majority of speech droplets were produced when the consonants "B", "P", "F", "V", "D", "T" and "S" were pronounced forcefully and with the lips wet; quiet, even speaking with the lips dry did not expel any droplets or expelled only a few.

The great majority of the expelled droplets were seen to disappear, presumably by evaporation to nuclei of subvisible size, within 1 second after leaving the mouth and while still suspended in the air within 4 feet in front of the mouth; this evaporation was most apparent in the 'clearing' of the droplet cloud produced by a sneeze. A smaller number of droplets, presumably of larger size, remained visible and were seen to lose their forward motion in the air within 4 feet in front of the mouth; these either fell slowly to the ground during 2 to 20 seconds, or fell only a large or small proportion of the distance to the ground and then ceased to fall, presumably having shrunk by evaporation to become nuclei. These droplet nuclei were watched while they drifted about with the air currents of the room. A few large "projectile droplets"/

droplets" were seen to fly forwards for 2 to 10 feet, curving downwards to reach the floor at about 1 second after their emission from the mouth.

(5) The maximum projection distance of the large "projectile droplets" was measured by exposing white paper on the floor to droplet spray produced with the mouth secretions colored by a dye. The furthest distance from the spray producer at which colored marks were found was 10½ feet for 'natural sneezing', 8½ feet for simulated coughing and 5 feet for speaking. In all cases, the majority of the expelled droplets fell to the ground within 4 feet of the spray producer.

(6) Four methods were used to estimate the numbers of droplets and nuclei produced by each different kind of expiratory activity:

i) counting colonies on blood agar plates exposed directly to spray in front of mouth and nose;

ii) counting by microscopical observation the deposit marks of dye-containing droplets caught on celluloid slides exposed directly to spray in front of mouth;

iii) counting colonies on blood agar plates exposed in slit sampler for collection of droplet nuclei from air of chamber contaminated with spray;

iv) counting by microscopical observation the dye-containing droplet nuclei on oiled slides exposed in slit sampler for collection of nuclei from air of a chamber contaminated with spray.

The/

The last mentioned method (iv) was a new method which was developed and used for the first time in the present investigation. It yielded much higher and more complete droplet counts than any of the other methods. In addition, it made possible microscopical observation and measurement of the droplet nuclei. Calculations from the observed size-distributions of the droplets and nuclei collected and from the computed bacteria-carrying percentages of droplets of each size, indicated that the probable counting efficiency of method (i) was 5-30%, of method (ii) 8-40%, of method (iii) 5-10%, and of method (iv) 75-97%. Use of methods (iii) and (iv) in parallel revealed that only about 5% (between 1 and 12%) of the microscopically visible droplet nuclei carried viable respiratory commensal bacteria. The droplet and nucleus counts obtained in the present study by methods (i), (ii) and (iii) were of the same order of magnitude as the counts obtained by previous investigators who used these methods.

The average rate of droplet expulsion observed was approximately 1000,000 droplets per 'natural sneeze', 1000 droplets per simulated cough, 1 droplet per spoken word, 1 droplet per minute of violent simulated laughing, 100 droplets per strong nasal expiratory "sniff", and 1 droplet per 3 minutes of nose breathing.

The number of droplets expelled by separate expiratory acts of the same kind varied very greatly according/

according to the violence of expiration and the wetness of the lips and front of mouth. A violent simulated sneeze expelled from 1500,000 to 16,000,000 droplets; a 'natural sneeze' from 65,000 to 3100,000 droplets; a simulated cough made with the mouth initially closed, from 490 to 52,000 droplets; a simulated cough made with the mouth kept well open, from 0 to 1100 droplets (frequently none); speaking loudly one hundred words, from 50 to 770 droplets; speaking quietly one hundred words, from 0 to 160 droplets; violent simulated laughing for one minute, from 0 to 12 droplets; a strong nasal expiration, from 0 to 1200 droplets; mouth breathing for one minute, 0 droplets; nose breathing for 5 minutes, from 0 to 6 droplets (frequently none).

In consideration of these droplet counts in relation to suggested yearly frequency rates for the performance of each kind of expiratory activity by a healthy person (e.g. a throat 'carrier' of a pathogenic bacterium), it is concluded that sneezing is by far the most important cause of droplet spray; the droplet output of 10 sneezes will probably exceed a person's yearly output of droplets by all other causes together, coughing, speaking, breathing and laughing.

Comparison of the counts obtained by the droplet-collecting methods (i and ii) with the counts obtained by the nucleus-collecting methods (iii and iv) showed that/

that a considerable proportion, if not the great majority, of the droplets expelled by each expiratory act were small enough to remain airborne as droplet nuclei.

(7) The sites of origin of droplets and nuclei were investigated in experiments with the throat or nose artificially contaminated with a culture of B.prodigiosus (Ser.marcescens). It was found that the great majority of the droplets and nuclei produced by sneezing, coughing and speaking, originated from the secretions of the front part of the mouth. A 'natural sneeze' which expelled probably about 1000,000 droplets, was found to expel directly from the nose between 0 and 5600 droplets (on average 250), of which between 0 and 360 (on average 56) were small enough to remain airborne as nuclei. A 'natural sneeze' was found to expel directly from the throat between 0 and 2300 droplets (on average 360), of which between 0 and 390 (on average 110) were small enough to become airborne nuclei. A simulated cough made with the mouth kept well open expelled directly from the throat between 0 and 279 droplets (on average 31), of which between 0 and 5 (on average 2) were small enough to remain airborne as nuclei. Speaking loudly words containing "K" and simulated laughing both expelled a few droplets directly from the throat. Nose breathing for 5 minutes expelled from the nose between 0 and 6 droplets (on average 2), of which some became nuclei.

(8)/

(8) The diameters of the large and medium sized droplets were calculated from the micrometrically measured diameters of 15,000 deposit marks of droplets caught on celluloid slides held at 6 inches in front of the mouth during sneezing, coughing or speaking with the mouth secretions colored by Congo red; the diameters of the spherical parent droplets were taken as half the diameters of the droplet deposit marks. The diameters of the medium sized and small droplets were calculated from the micrometrically measured diameters of 25,175 droplet nuclei collected by the slit sampler on to oiled slides from the air of a chamber contaminated by sneezing, coughing or speaking with the mouth secretions colored by Congo red, and the mouth usually situated at 5 feet above the floor; the diameters of the parent droplets before evaporation were calculated as 4 times the diameters of the Congo red-containing droplet nuclei. By appropriate combination of the two series of measurements a composite size-distribution series was constructed for all the droplets expelled, large, medium and small. These calculations showed that the droplets expelled by sneezing, coughing or speaking, at the moment of their emission from the mouth, ranged in diameter from about 1 to 2000 microns. Practically this full range of variation in size was shown by the droplets emitted on any one occasion by a single expiratory act. There was not much difference between the sneeze droplets, the/

the cough droplets and the speech droplets; in each case, the great majority (60-85%) were between 2 and 25 microns in diameter; the commonest diameters among the sneeze droplets, 2 to 5 microns, were rather smaller than the commonest diameters among the cough droplets and speech droplets, 5 to 10 microns. It was calculated that about 99% of sneeze droplets, 92% of cough droplets and 87% of speech droplets were smaller than 100 microns in diameter and thus were small enough to remain airborne as droplet nuclei.

The diameters of the 25,175 Congo red-containing droplet nuclei collected by the slit sampler, which must have been 25-50% larger than natural because of their dye content, varied from a ¼ to 42 microns; the majority were between ½ and 6 microns; the commonest were about 1 to 2 microns. The diameters of 376 dye-containing nuclei collected by sedimentation on to slides exposed on the floor, ranged from ½ to 35 microns. Only 8 out of the total 25,551 nuclei were larger than 25 microns in diameter; the exceptional size of these 8 nuclei was probably due to an unusually large solid content. Accepting 25 microns as the usual upper size limit for droplet nuclei, it was calculated that a droplet of 100 microns in diameter (25 x 4) must be about the largest droplet which after emission at 5 feet above the floor can remain airborne as a droplet nucleus.

(9)/

(9) The proportion of droplets in each size group which will contain one or more viable micro-organisms of a given kind, commensal or pathogenic, was computed for certain likely salivary concentrations of micro-organisms (as "viable microbial units" per milliliter), on the assumption that the distribution of these among droplets of each size would be of the Poisson type. The calculations showed that only a small proportion (7% to 24%) of the droplets small enough to remain airborne as nuclei will contain one or more viable commensal bacteria even when, as is usual, large numbers of these commensal bacteria are present in the saliva (e.g. 30,000,000 per milliliter). A very small proportion (e.g. 0.0006% to 15%) of the droplets small enough to remain airborne as nuclei will contain one or more viable pathogenic bacteria, since the saliva seldom contains more than small numbers of these (e.g. 1000 to 1000,000 per milliliter). These calculated percentages of all nuclei which will be infected with with commensal or pathogenic bacteria, were found to be in agreement with the figures obtained by direct observation of infected nuclei both in the present investigation and the investigations by previous workers.

(10) The duration of air carriage of differently sized droplet nuclei was observed by repeated slit sampler examinations of the droplet nucleus content of the air of a practically unventilated chamber following contamination/

contamination by sneezing or coughing. The 90%-disappearance time of microscopically visible nuclei of all sizes was between 2 and 11 hours, on average 5 hours, in a small (70 cu.ft.) sealed chamber, and between 20 and 60 minutes, on average 45 minutes, in a larger (1700 cu.ft.) chamber which was not so well sealed against ventilation. When the chamber air was circulated vigorously by continuous running of a fan, disappearance of nuclei was hastened between 2-fold and 4-fold; the 90%-disappearance time was between 10 and 30 minutes.

In the unventilated test chambers following sneezing, the maximum duration of air carriage was 30 hours for microscopically visible nuclei about 1 micron in diameter, 2 hours for 5-micron nuclei, 20 minutes for 10-micron nuclei, 2 minutes for 20-micron nuclei, 8 hours for nuclei containing B.mesentericus spores which had been added to the saliva to the extent of about 1000,000,000 per milliliter, and 2 hours for nuclei containing viable commensal bacteria of which the saliva contained about 30,000,000 per milliliter. Almost certainly the number of viable pathogenic bacteria in saliva will never exceed the usual number of viable commensal bacteria; this suggests that the air of an unventilated room will never remain infected with nuclei containing pathogenic bacteria for longer than 2 hours after droplet spray production. When, as is usual, the infected saliva contains only small numbers/

numbers of pathogenic bacteria (e.g. 1000 to 1000,000 per milliliter) and when the room is ventilated to the usual extent of between 1 and 10 overturns per hour, droplet nucleus air infection is unlikely to persist for longer than a few minutes.

Sedimentation rates for droplets and nuclei of different size were calculated from the above observations and from observations of the time between a sneeze and the arrival of droplets on slides exposed on the floor. These "observed" sedimentation rates approximated closely to the sedimentation rates which were calculated by Wells (1934) according to Stokes' law.

(11) Reinfection of the air with droplet nuclei raised from the chamber floor and walls as the result of a person's bodily movement, was observed in two special experiments. A chamber was contaminated by sneezing with the saliva heavily inoculated with B.mesentericus spores. After the air had become free of the spore-containing nuclei by sedimentation, it was found to become reinfected with this indicator organism when a person entered the chamber and "marched" for ten minutes.

(12) The evaporation of large saliva droplets hanging from fine glass fibres and of smaller saliva droplets lying on the surface of slides, was observed microscopically and timed. Comparison of the observed

evaporation times with the observed falling times led to the same conclusion as was reached by Wells (1934) on a theoretical basis, namely that droplets of 100 microns in diameter are about the largest droplets capable of remaining airborne as droplet nuclei.

Momentary air sampling revealed the presence, within 1 to 2 seconds after a sneeze, of fully evaporated droplet nuclei of all sizes up to about 12 microns; presumably these originated from droplets up to about 50 microns.

(13) From these various observations, conclusions were drawn about the sanitary significance of droplet spray, about the extent and limits of the danger of infection from droplet spray.

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Part 3

EXPERIMENTAL INVESTIGATION OF THE AMOUNT, PERSISTENCE AND CAUSATION OF AIR INFECTION WITH DUST FROM SKIN AND CLOTHING, AND OF THE EFFICACY OF DIFFERENT GOWNING METHODS IN PREVENTING SUCH DUSTBORNE INFECTION OF AIR.

CONTENTS OF PART 3

Section 1: Introduction.

Bacteria can enter the air in two ways, in droplet nuclei or on dust particles. Droplet nucleus infection was the subject of the investigation just described (Part 2); the investigation which is now described, was concerned with dustborne infection.

For a long time it has been considered that the raising of dust contaminated with dried respiratory secretion may be an important cause of air infection (e.g. Cornet, 1889). Attention has been centered on floors, furniture and bedclothes as sources of infected dust; undoubtedly, these will be of major importance in spreading infection within the home, school, common hospital-ward and residential institution. Less attention has been paid to the skin and clothing of infected persons as sources of dustborne air infection; yet these have a special importance in that they constitute "mobile" and "self-replenishing" sources of infection which are continually subject during bodily activity to frictions and movements likely to cause liberation of dust. Observations such as those of Hare (1941) and of Hamburger, Green and Hamburger (1945) on throat and nose carriers of Strept.pyogenes, and those of Augustine (1929) on patients with pulmonary tuberculosis, have shown that persons with respiratory tract infection usually accumulate the specific pathogenic organism in large numbers on their skin and clothing, probably by contamination with secretion/

secretion and droplets from mouth and nose.

The present experimental investigation had a twofold purpose. Firstly, measurements were made of the amount of dust liberated into the air from a person's skin and clothing during different kinds of bodily activity, and of the duration of air carriage of these dust particles; these measurements were required for assessing the relative importance of dustborne infection and droplet nucleus infection. Secondly, observations were made of the efficacy of different modes of gowning in preventing this air infection with dust from the skin and clothing; apart from the importance of such gowning in surgical practice and in bacteriological air sampling, measurements of the air infection produced by the test subject while wearing a sterile dustproof gown were necessary as a control for the measurements of the air infection produced by the subject while wearing his ordinary clothes uncovered.

All experiments were made according to the same plan. A person dressed in a particular way entered a small unventilated chamber and remained in this for ten minutes undertaking a standard form of bodily activity. Air samples of known volume were withdrawn from the chamber before, during and after the ten-minute period of occupancy and activity. These samples were taken on to culture plates in a Bourdillon slit sampler for examination of their content of bacteria-/

bacteria-carrying particles. The colony count obtained on each plate represented the number, in the air sample, of dust particles carrying viable aerobic bacteria. Thus, a purely bacteriological method was used for measuring the dust liberated from the skin and clothing. A bacteriological method has certain limitations. For instance, probably only a small proportion of the dust particles carry viable bacteria (saprophytes or commensals), so that only a small proportion can be counted. However, the cultural method was preferred to other methods for measuring the amount of dust, for instance the microscopical methods, because (1) it is easy and rapid, (2) it allows measurement of dust originating from skin and ordinary non-sterile clothes as distinct from dust originating from sterile gowns, and (3) it reveals the dust particles which are large enough to carry bacteria but not the more numerous dust particles which are too small to carry bacteria and thus not of any sanitary significance.

Great care was given to designing the experiments in such a way that infection of the air in the test chamber could only occur as result of liberation of dust from the skin and clothing of the subject. Droplet-spray was avoided by the subject keeping his mouth closed while he was present in the chamber. Raising of dust from walls and floor was prevented by oil treatment of these surfaces. With these precautions./

ions, it was considered justifiable to assume that nearly all the observed amount of bacterial contamination of the chamber air was due to liberation of dust from the subject's skin and clothing.

This investigation (Part 3) was carried out in collaboration with Dr. A.T. Wallace.

Section 2: Test chamber and air sampler.

The experiments were made in a test chamber of 100 cubic feet in capacity (8 ft. high x 3½ ft. x 3½ ft.). The chamber had a close fitting door and was quite unventilated apart from the narrow slit between the edge of the door and its frame. The interior surfaces of the chamber, the walls and floor, were soaked repeatedly with spindle oil to minimise air infection by liberation of dust from them. During occupancy, the temperature in the chamber was usually between 55 and 60 deg.F. (13-16 deg.C.) and the relative humidity was between 50% and 90%. It was not considered necessary to employ an electric fan to obtain even distribution of dust particles throughout so small a chamber; the movements of the test subject were thought to effect a sufficient mixing of the air.

The air was examined by use of a slit sampler constructed according to the design of Bourdillon, Lidwell and Thomas (1941). The sampler was situated outside the chamber and the person operating it also remained outside. Air was withdrawn through the wall of the chamber to the sampler intake by a short, wide and slightly curved glass tube. The entrance to this tube within the chamber was 3 feet from the floor and was screened from above to prevent dust falling directly into it. The slit sampler was operated at a slit-plate distance of 2 millimeters and at an air-intake rate of 1 cubic foot per minute.

The chamber and sampler are shown in Figure 10.

Section 3: Methods of culture and colony-counting.

The culture plates exposed in the slit sampler contained either ordinary blood agar or heated blood agar. The ordinary blood agar consisted of 1% meat extract, 1% peptone, ½% sodium chloride, 2% agar and 5% horse blood; the blood was added to the nutrient agar when the latter was at a temperature just above its setting point. Heated blood agar consisted of this ordinary blood agar heated briefly to nearly 100 deg.C. so as to "chocolate" the blood. Both media were regarded as suitable for growth of the majority of the saprophytic and commensal bacteria from clothing and skin. Heated blood agar was used when it was desired to make a separate count of Strept.viridans, since the alpha haemolytic reaction, the greening, was more distinct on this medium.

The culture plates were incubated aerobically at 37 deg.C. for 18 to 24 hours; longer incubation was avoided since it resulted in frequent spoiling of the plates by spreading colonies. After scoring of the agar surface by parallel cuts with a scalpel, the colonies were counted with the aid of a binocular plate microscope (x 20); the microscope was required for sure recognition of the smaller colonies.

Section 4: Test subjects and bodily activities.

Each experiment was made with one out of eight healthy men who were not "carrying" pathogenic bacteria (subjects D, W, A, K, M, S, J, L).

The test subject entered the chamber quietly, closed the door and, while keeping his mouth closed to avoid droplet-spray production, undertook a standard form of activity during 10 minutes occupation of the chamber; he then left the chamber and closed the door.

The standard activities tested were:

(1) "Standing Motionless", avoiding as far as possible the slightest movement;

(2) "Undressing and Dressing", taking off all clothes and replacing them once during the 10 minutes;

(3) "Brushing Clothes", applying 40 strokes with a sterilised brush to the front of the jacket and trousers, 20 strokes in each half of the 10-minute period;

(4) "Operating", making certain movements of the arms and body, without movement of the feet. In the first series of experiments (No.s 1-68), the test subject stood fairly still for most of the 10 minutes, but on four occasions, at equal intervals during the 10-minute period, made movements in a pretence at changing culture-plates in an imaginary slit sampler, bending the body and reaching out with the arms. In the third series of experiments (No.s 81-116), the test subject made a standard set of arm and body movements designed

to/

to represent the movements of a surgeon while carrying out an operation: 12 swings of the extended arms through 180 deg., 24 times reaching out an arm sideways to full length and retracting it, 36 bendings of the body through 45 deg. to right, left, or forwards, 48 times reaching forwards with both arms and withdrawing them to the body, and 48 times rocking the shoulders with alternate advancement of right and left arms. These surgical "operating" movements were made deliberately and not violently; their performance occupied most of the 10-minute period;

(5) "Marching", walking without progression continuously throughout the 10-minute period, raising the feet and swinging the arms;

(6) "Running", running without progression continuously throughout the 10-minute period, raising the feet and swinging the arms.

Section 5: Modes of dress and gowning.

(1) "Ordinary Clothes":- The test subject, a male adult, was dressed in his usual clothing, which consisted of undergarments and either a lounge suit or a combination of jacket, trousers and woollen pullover. These clothes were, of course, non-sterile; they had not been treated in any special way. Prior to the experiment, the outer garments had been in normal use for a few weeks since last being washed or cleaned. In the first series of experiments (No.s 1-68), the test subject wore his usual non-sterile outdoor shoes, and did not wear sterile gloves, cap or mask (Figure 14a). In the second series of experiments (No.s 69-80), the test subject wore sterile rubber shoes instead of his usual shoes, and wore also sterile rubber gloves, a sterile surgeon's-cotton-cap, and a sterile surgeon's-mask of gauze and cellophane (Figure 16a).

(2) "Sterile Clothes":- The test subject removed all his usual clothes, including socks and undergarments, and put on a sterile cotton shirt with sleeves reaching to the elbow, a sterile pair of long cotton trousers with legs reaching to the ankles, sterile cotton socks, sterile rubber shoes, sterile rubber gloves, sterile cap and sterile surgical mask (Figure 17a). The subject changed into these sterile clothes just before the experiment; while changing, he stood on a sheet of sterile paper to minimise contamination of the garments from the floor. "Sterile Clothes" were worn in the third/

third series of experiments (No.s 81-116).

(3) "Surgical Gowning":- Over "Ordinary Clothes" or over "Sterile Clothes", the subject in certain experiments wore a sterile surgeon's-gown of the usual pattern; this gown was made of thin close-woven cotton cloth, was long and loose, was tied together with tapes at the back, reached to below the knees where it was quite open, and possessed sleeves which reached to the wrists. Sterile rubber gloves were worn and the gown cuffs were tucked inside these. A sterile cotton cap and a sterile gauze-cellophane mask were also worn. In the first series of experiments (No.s 1-68), the surgical gown was worn over "Ordinary Non-sterile Clothes"; disinfected long rubber boots were worn which covered the lower parts of the trouser legs (Figure 14b). Similarly, in the second series of experiments (No.s 69-80), the sterile surgical gown was worn over "Ordinary Clothes" (Figure 16b). In the third series of experiments (No.s 81-116), the sterile surgical gown was worn over "Sterile Clothes" (Figure 17b).

(4) "Dustproof Gowning":- Over "Ordinary Clothes" or over "Sterile Clothes", the test subject in certain experiments wore a sterile dustproof gown. This dustproof gown was a one-piece costume of heavy close-woven cotton twill. It covered the body, arms and legs. The pocket slits were sewn up. A zip fastener was fitted instead of buttons to close the front. Elastic bands were put over the sleeve cuffs so that these/

these gripped the wrists closely. A 'sock' of heavy close-woven canvas was sewn to the bottom of each trouser leg to form a complete covering for the foot and non-sterile shoe. Close fitting at the neck was ensured either (a) by having sewn on to the gown collar edge a soft cotton cloth extension which was tucked over and down inside the collars of the jacket and shirt beneath, or (b) by having a soft bulky muslin pad sewn along the whole length of the inner surface of the gown collar and by making this grip the neck closely by tying together with tapes the free front ends of the gown collar. The "Ordinary Clothes" or "Sterile Clothes" were thus completely covered without gaps at the feet, wrists, neck or elsewhere for escape of dust-laden air. "Dustproof Gowning" is illustrated in Figures 14c, 16c and 17c.

(5) Sterilisation Methods:- The sterile clothes, the surgical gown, the cap and the mask were sterilised by autoclaving at 120 deg.C. for 15 minutes. The rubber shoes were sterilised by steaming and the rubber gloves by boiling for several minutes.

Section 6: Plan of experiment and sampling.

Each experiment of the first series (No.s 1-68) was carried out as follows. In the "Control Period", just prior to occupation of the chamber, two successive 10-cubic feet samples were taken. During the 10-minute "Activity Period", while the chamber was occupied, four successive 2-cubic feet samples were taken. During the four hours after termination of occupancy and activity, the "Die-away Period", further samples were taken at intervals: 2-cubic feet samples for the first hour and 10-cubic feet samples thereafter.

In experiments of the other two series (No.s 69-80, and No.s 81-116), fewer samples were taken; the first of the two samples in the Control Period and all except the first sample in the Die-away Period were omitted.

Section 7: Contamination of air by subject wearing ordinary non-sterile clothes not covered with a gown.

The detailed results of the first series of experiments (No.s 1-68), in which the test subject wore ordinary non-sterile clothes (as in Figure 14), are shown in Tables 56, 57, 58 and 59. Seventeen experiments were made with each of four subjects (D, W, A and K). The experiments in which a gown was not worn over the ordinary clothes, are summarised in Table 60a and Graph 8; these show the average amount of air contamination which was produced by each different kind of bodily activity.

While the chamber was unoccupied there was little contamination of the air; the average of the control-period observations was 2 bacteria-carrying particles per cu.ft. of air. Bacterial contamination was always increased on occupation of the chamber, the amount depending on the kind of activity undertaken by the occupant. Considered in terms of the average number of bacteria-carrying particles per cu.ft. of air during the "activity period", the different kinds of bodily activity gave increasing amounts of air infection in the following order: "standing motionless" gave 9 per cu.ft., "operating" gave 129 per cu.ft., "brushing clothes" gave 501 per cu.ft., "marching" gave 837 per cu.ft., and "undressing and dressing" gave 1672 per cu. ft.. Apparently the amount of bacterial contamination of the air varied in proportion to the amount and vigour

of the friction and movement to which the skin and clothing were subjected.

Because the dust particles settle at different rates, and some very rapidly, it is impossible to calculate from the number found per cu.ft. the exact total number put into the air during the 10-minute "activity period". However, a minimum can be calculated from the first observation made in the "die-away period". This sample was not started until one minute after the occupant had left the chamber. It is assumed that by this time the dust particles had become uniformly dispersed throughout the entire 100 cubic feet of the chamber; dispersion was aided by the air disturbance caused when the occupant opened and shut the door on leaving the chamber. The number of bacteria-carrying dust particles per cu.ft. found in the first sample of the "die-away period" was multiplied by 100 to give the total number in the chamber at that time. These calculated minimum numbers of bacteria-carrying particles capable of remaining airborne for more than 1 minute, which were liberated by the various bodily activities, are shown in the last column of Table 60a; the average number liberated was 800 by 10 minutes of "standing motionless", 11,600 by 10 minutes of "operating", 49,500 by "brushing clothes" with 40 strokes during 10 minutes, 67,100 by 10 minutes of "marching", and 157,100 by "undressing and dressing" once during 10 minutes. If the amount of the standard "operating"/

"operating" activity is about equivalent to the average activity of daily life, the air must normally become contaminated from the skin and personal clothing at the rate of about 1,000,000 bacteria-carrying dust particles per day (i.e. 14½ hours "operating" per day).

Section 8: Contamination of air by subject wearing ordinary non-sterile clothes covered with a sterile gown.

The results of the experiments recorded in Tables 56, 57, 58 and 59, and summarised in Table 60b and Graph 9 (i.e. experiments No.s 1-68), showed that contamination of air with dustborne bacteria from the skin and clothing was only slightly reduced by "surgical gowning", but was greatly reduced by "dustproof gowning". With "surgical gowning" the average amount of bacterial contamination of the air during "operating" was 57%, and during "marching" 59%, of that found in the comparable experiments without gowning. With "dustproof gowning" the average amount of bacterial contamination of the air during "operating" was 12%, and during "marching" 4%, of that found in the comparable experiments without gowning.

Wearing of the sterile surgical gown gave very poor protection against infection of the air, probably because the dust particles from the skin and clothing were freely expelled through the gaps at the back of the gown where it was tied with tapes and through the wide opening at the foot. The high degree of protection afforded by wearing of the sterile dustproof gown was due presumably to this garment completely covering the non-sterile clothes and the friction-exposed areas of skin, without leaving any gaps for escape of dust-laden air. The hands, the skin of the face and the hair of the head did not appear to give

off/

off an important amount of dust, presumably because they were not exposed to friction; further decrease in air infection was not obtained by covering or oiling these parts.

The importance in "dustproof gowning" of avoiding even small gaps in the covering, was demonstrated in a second series of experiments (No.s 69-80) which are recorded in Table 61; these were made with two test subjects (D and M). The amount of bacterial contamination of the air which was produced by "marching" was observed for four states of dress: for (a) the subject wearing "ordinary clothes" without gowning (Figure 16a), (b) the subject wearing "surgical gowning" over his ordinary clothes (Figure 16b), (c) the subject wearing the complete, standard "dustproof gowning" over his ordinary clothes (Figure 16c), and (d) the subject wearing <u>incomplete</u> "dustproof gowning" over his ordinary clothes. This <u>"incomplete dustproof gowning"</u> differed from the standard "dustproof gowning" in two respects: the gown collar lacked a pad or extension to make it fit the neck closely, and there were not the canvas 'socks' sewn to the ends of the trouser legs; sterile cotton socks were worn and the ends of the trouser legs were tucked inside these. With this <u>"incomplete dustproof gowning"</u> the amount of air contamination produced by "marching" was much greater, 2 to 6 times greater, than with <u>"complete dustproof gowning"</u>, although still less than

with/

with "surgical gowning". Probably the escape of dust-laden air through the loose neck of the gown constituted the main deficiency of the "incomplete dustproof gowning". The results of these experiments (No.s 69-80) show how necessary for proper protection against infection of the air is the avoidance of any gaps in the gown and the greatest exercise of care in donning and fitting the gown.

Section 9: Duration of air-carriage of bacteria-carrying dust particles from skin and clothing.

The results of the experiments with subjects wearing ordinary non-sterile clothes (i.e. No.s 1-68; Tables 56-59) show that bacterial contamination of the air remained appreciably above the control level usually for more than 20 minutes and sometimes for as much as 2 hours. Graph 10 shows the long persistence of air infection following "operating" in one experiment. For the experiments with a maximum recorded air contamination of over 1000 bacteria-carrying particles per cu.ft., the average time until disappearance of all but 10% of these particles was 35 minutes. Since much importance was attached to this considerable persistence of dustborne bacterial contamination of the air, a possible but unlikely cause of error was carefully considered. Infected dust particles which were drawn into the air sampler intake-tube while sampling during the activity period, might adhere to the walls of the tube and then, during the taking of later samples, become free and pass into the sampler. To exclude this possibility, experiments were made in which the air was heavily infected by "marching" and, to avoid contamination of the intake-tube, not any samples were taken during the activity period or during the die-away period until 30 minutes after the test subject had left the chamber. The samples taken after this time showed amounts of air infection/

infection which were much above the control level (37, 38, 71, 33 and 86 bacteria-carrying particles per cu.ft. in five experiments); this confirmed that infected dust may remain airborne for a considerable time.

Section 10: Dissemination of respiratory tract bacteria in skin and clothing dust.

Since the test subjects were not infected with pathogenic organisms, the bacteria recovered from the air were exclusively non-pathogenic. They were mainly saprophytic species whose normal habitat is the skin or clothing. Staph.albus, diphtheroid bacilli, Micrococci and fungi were the commonest species.

Proof was desired that persons with respiratory infection would disseminate pathogenic organisms on a proportion of the dust particles which they liberate from their skin and clothing. The presence in air of Strept.viridans has been suggested as a reliable indicator of contamination from the upper respiratory tract (Gordon, 1904; Wells and Wells, 1936). All persons carry large numbers of Strept.viridans in the saliva and throat secretions; Gordon found from 10,000,000 to 100,000,000 per ml. of saliva.

Therefore, in 61 of the experiments with persons wearing ordinary non-sterile clothes (47 of those in Tables 56-61, 5 others with subject M., and 9 with another subject, S.) heated blood agar plates were used for sampling and separate counts were made of the colonies which were alpha haemolytic and resembled Strept.viridans. A few such alpha haemolytic colonies were found in almost every experiment with each of six test subjects. On the plates exposed in the 61 experiments, which bore a total of 173,103 colonies of bacteria/

bacteria of all kinds, there were 586 (i.e. 0.34%) which resembled Strept.viridans colonies and were surrounded by zones of greening. However, on microscopical examination of smears of blood-agar and nutrient-broth subcultures of 295 of these alpha haemolytic colonies, chain formation and streptococcal morphology was found in only 29; that is, only 0.033% of the colonies of all species were alpha streptococci. The remaining 266 of the alpha haemolytic colonies which were examined microscopically, consisted of diphtheroid bacilli or, more commonly, of Gram-positive cocci grouped in pairs, tetrads or clusters. These latter apparently corresponded to the "putative streptococci" which Buchbinder, Solowey and Solotorovsky (1938) found comprising 60% of the green-producing streptococcus-like colonies obtained in sampling air, and which they regarded as probably streptococci derived from the respiratory tract but altered in morphology as a result of their aerial environment. This assumption of Buchbinder and his colleagues seems rather improbable. It seems more likely that the "putative streptococci" are not true streptococci but belong to the genus Micrococcus. Their original habitat may be the skin, clothing or other surface outside the respiratory tract. Their presence in air certainly can not be regarded as proof of contamination from the respiratory tract. Thus, in the present experiments, the extent of bacterial contamination/

contamination of the air from the respiratory tract via clothing is indicated not by the 0.34% of airborne bacteria which gave streptococcus-like colonies with greening but by the 0.033% which showed chain-formation on microscopical examination.

The data concerning Strept.viridans and other alpha haemolytic bacteria are summarised in Table 63. Of the six test subjects, three distributed true alpha streptococci into the air in dust from their skin and clothing, on at least some of the occasions on which they were examined. It may be noted that 19 of the true alpha streptococcus strains which were thus recovered from the air, were tested for mucoid colony formation on the 5% sucrose medium of Sherman, Niven and Smiley (1943); 5 of the strains formed mucoid colonies and were classed as Strept.salivarius in the sense of these authors.

It may be concluded that bacteria from the respiratory tract normally contaminate the skin and clothing and, in small numbers, become liberated into the air on dust particles.

Section 11: Distribution into air of indicator organism from chamber floor and walls.

On many occasions during the 15 months of the investigation, the floor and walls of the test chamber were soaked with spindle oil so as to prevent dust being raised from them during "marching" and "running". Only in the certainty that there was not a considerable amount of air contamination from the floor and walls, could the observed air contamination be attributed to liberation of dust from the skin and clothing.

Some special experiments were made to test the efficacy of the 'oiling' method of dust suppression. Use was made of B.mesentericus as an indicator of contamination from the floor and walls. Being a spore-former, this bacillus could survive for a long time on the floor and walls. The characteristic colonies of the bacillus were easily recognised on the culture plates, being unlike the colonies of any of the bacteria normally found in the air. Except in these special experiments when it was introduced artificially, B.mesentericus was never found in the chamber air.

The chamber floor and walls were contaminated with B.mesentericus in the following manner. The observer took into his mouth about 1 ml. of a dense aqueous suspension of an old, sporing culture of B.mesentericus; he then gave several violent simulated sneezes into the test chamber. The door of the chamber was closed and the chamber was left undisturbed for one or more days.

During/

During this time all the B.mesentericus-containing droplet nuclei settled out of the air on to the floor and walls of the chamber. At the end of the settling period the air was demonstrated by sampling to be free from B.mesentericus.

After the chamber floor and walls had thus been contaminated with B.mesentericus spores, experiments were made with "marching" by a test subject. The culture plates were examined specially for colonies of B.mesentericus and these were counted separately from the other colonies. It was found that, in spite of the 'oiling' treatment, a considerable number of B.mesentericus were raised into the air by "marching". The average activity-period counts in 7 experiments were 37, 78, 12, 59, 41, 29 and 38 B.mesentericus particles per cu.ft. (average, 42 per cu.ft.). It is thought that these figures represent a much higher degree of air contamination from the floor and walls than would normally occur, since these surfaces had been artificially contaminated with an enormous number of small infected particles. Yet even this artificially exaggerated air contamination from the floor and walls corresponded to only a small fraction of the total air contamination observed in experiments with "marching" by a subject in "ordinary non-sterile clothes" or in "surgical gowning" (Table 60b). In such experiments, therefore, serious error can not have been incurred by disregarding contamination from the floor/

floor and walls, and attributing all the observed air infection to contamination from the skin and clothing. On the other hand, the amounts of air contamination observed in experiments with "marching" by a subject in "dustproof gowning" (Table 60b) were of the same order as, or even less than, the amounts of air contamination with B.mesentericus from the floor and walls as observed in these special experiments. Hence the possibility must be admitted that a substantial part of the air contamination observed in experiments with "marching" and "running" by a subject in "dustproof gowning", was due to raising of dust from the floor and walls.

Apart from the value of these special experiments in controlling the experimental method, they afford an interesting demonstration of air infection by the reintroduction of sneeze droplet nuclei into the air after their settlement on to the floor.

Section 12: Contamination of air by subject wearing only sterile clothes.

The experiments made with subjects dressed exclusively in sterile clothes (No.s 81-116) were mainly the work of Dr. A.T. Wallace. The results of these experiments are not given in detail in this report, but a summary of the results is given in Table 62 in order to allow comparison with the experiments made on subjects wearing ordinary clothes (see also Graph 9).

In the experiments made without a gown being worn over the sterile clothes, a considerable amount of air infection was caused; presumably the bacteria-carrying dust particles originated directly from the skin or from the inner surfaces of the "sterile clothes" after contamination of these from the skin. Out of experiments with 4 subjects (D, W, J and L) the overall average activity-period air infection levels were 83, 200 and 559 bacteria-carrying particles per cu.ft. for, respectively, "operating", "marching" and "running" in "sterile clothes". These levels may be compared with the corresponding average levels of 129 and 837 bacteria-carrying particles per cu.ft. for, respectively, "operating" and "marching" in "ordinary non-sterile clothes". The measurements for "operating" in "sterile clothes" are not strictly comparable with the measurements for "operating" in "ordinary non-sterile clothes", since in the former case the "operating" consisted/

consisted of a greater number of movements than in the latter case (see Section 4). On the other hand, the experiments on "marching" in "sterile clothes" were entirely comparable in amount of activity with the experiments on "marching" in "ordinary non-sterile clothes"; the comparison of the two series showed that the substitution of sterile for non-sterile clothes reduced the amount of air infection with personal dust to about a quarter (200 being 24% of 837).

From the point of view of aseptic practice in surgery, great interest attached to the amount of air infection which was caused by 10 minutes of "operating" by a person in "sterile clothes". For this reason and because there may be great variation between different persons and occasions, the four experiments recorded in Table 62 were supplemented by further experiments on the same and other subjects. The average activity-period air infection levels for 9 experiments on "operating" in "sterile clothes" were, per cu.ft., 222, 605 and 246 for subject D., 13 for subject W., 49 for subject J., 46 and 9 for subject L., 64 for subject Sw., and 8 for subject H.. The calculated total numbers of bacteria-carrying particles present in the 100-cu.ft. chamber at between 1 and 3 minutes after "operating" were 20,500, 70,500 and 21,500; 1300; 4800; 6700 and 800; 6000; and 900.

The effect of "surgical gowning" and "dustproof gowning" on the amount of bacterial contamination of the/

the air by persons wearing "sterile clothes", was similar to the effect observed in the case of persons wearing "ordinary non-sterile clothes". As compared with the overall average activity-period air infection levels produced by persons wearing "sterile clothes" not covered with a gown, "surgical gowning" reduced the amount of air infection produced by "operating" to 75%, that produced by "marching" to 71% and that produced by "running" to 46%; these are reductions which may be regarded as inadequate and valueless. On the other hand, "dustproof gowning" effected much greater reductions, reducing the amount of air infection produced by "operating" to 13%, that produced by "marching" to 16%, and that produced by "running" to 7%. These reductions are shown in Table 62 and Graph 9.

It is apparent that the wearing of a sterile operating suit does not render unnecessary proper dustproof gowning, nor justify use of the inefficient method of gowning usually practised by surgeons.

Table 56:- Number of Bacteria-Carrying Dust Particles per Cubic Foot of Air in a 100 Cu.Ft. Chamber while Various Bodily Activities Undertaken by Subject D. with and without Gowning, as in Figure 14.

Activity:-	Standing Motionless			"Operating" (Arm & body movements only)						"Marching" (Swinging arms & raising feet)						Undressing & Dressing	Brushing Clothes
Gowning:-	No Gown	Surg-ical	Dust-proof	No Gown		Surgical Gowning		Dust-proof Gowning		No Gown		Surgical Gowning		Dust-proof Gowning		No Gown	No Gown
Culture Medium	BA	BA	BA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	BA
Experiment No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Control Period:																	
0 - 10 min.	6	4	3	1	1	2	1	1	1	1	3	2	2	2	2	1	3
11 - 21 min.	5	4	4	1	2	2	1	2	1	1	4	1	2	2	1	2	2
Activity Period:																	
22 - 24 min.	13	13	15	74	167	74	60	18	12	528	1332	527	366	17	35	858	815
24.7-26.7min.	11	18	7	142	307	114	95	22	16	1215	1953	986	865	29	37	2814	845
27.3-29.3min.	9	10	5	223	368	207	118	24	25	1233	2002	1201	864	45	53	3340	765
30 - 32 min.	12	9	5	240	387	201	139	25	26	1321	1683	1108	914	42	49	2485	1125
Die-away Period:																	
33 - 35 min.	9	11	6	181	250	86	100	21	21	928	1139	850	675	33	43	1545	839
37 - 39 min.	16	7	9	126	203	64	72	23	19	819	808	556	457	23	34	1623	468
42 - 44 min.	13	10	11	112	135	53	38	22	17	450	686	354	516	17	28	1237	259
52 - 54 min.	14	14	8	71	31	44	22	11	10	268	321	204	213	14	21	645	115
72 - 74 min.	11	12	24	30	9	19	8	8	5	140	122	82	84	9	12	212	21
92 -102 min.	7	4	-	21	7	13	2	7	4	96	59	35	45	5	7	96	11
152 -162 min.	4	4	-	12	4	8	1	5	1	43	30	13	14	2	3	30	6
212 -222 min.	2	3	-	11	3	5	1	3	1	22	10	8	3	2	1	11	6
272 -282 min.	-	-	-	-	1	-	1	-	1	9	8	4	3	3	1	5	4

See text for explanation of legends. BA: blood agar. CA: chocolate agar. - means no sample.

Table 57:- Number of Bacteria-Carrying Dust Particles per Cubic Foot of Air in a 100 Cu.Ft. Chamber while Various Bodily Activities Undertaken by Subject W. with and without Gowning as in Figure 14.

Activity:-	Standing Motionless			"Operating" (Arm & body movements only)						"Marching" (Swinging arms & raising feet)						Undressing & Dressing	Brushing Clothes
Gowning:-	No Gown	Surg-ical	Dust-proof	No Gown		Surgical Gowning		Dust-proof Gowning		No Gown		Surgical Gowning		Dust-proof Gowning		No Gown	No Gown
Culture Medium	BA	BA	BA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	BA
Experiment No.	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Control Period																	
0 - 10 min.	3	3	1	1	1	1	2	3	1	1	3	1	5	1	1	1	2
11 - 21 min.	2	5	1	2	1	4	2	4	2	0	4	1	7	1	2	1	2
Activity Period																	
22 - 24 min.	8	19	4	20	21	15	33	11	3	161	205	63	118	24	15	234	34
24.7-26.7min.	6	8	6	22	33	16	51	8	11	317	272	159	220	38	26	631	80
27.3-29.3min.	6	10	8	20	43	18	44	9	12	322	337	220	259	31	-	1791	137
30 - 32 min.	9	11	5	29	49	17	63	17	11	350	325	194	230	39	36	1708	186
Die-away Period																	
33 - 35 min.	6	6	10	25	48	19	45	11	10	247	246	193	156	38	35	1080	156
37 - 39 min.	5	8	4	28	33	13	38	12	10	123	201	134	140	26	24	739	104
42 - 44 min.	6	8	5	18	17	13	34	8	3	46	111	121	99	16	32	634	59
52 - 54 min.	3	8	5	17	12	9	21	10	4	13	65	99	54	17	21	343	49
72 - 74 min.	3	-	2	8	4	9	14	11	4	3	27	47	42	9	14	271	23
92 -102 min.	-	-	-	-	1	-	8	-	1	1	18	31	21	8	8	122	12
152 -162 min.	-	-	-	-	1	-	6	-	1	-	5	-	7	4	-	45	6
212 -222 min.	-	-	-	-	1	-	2	-	0	-	2	-	5	2	4	19	3
272 -282 min.	-	-	-	-	1	-	2	-	1	-	2	-	-	-	-	5	2

See text for explanation of legends. BA: blood agar. CA : chocolate agar. - means no sample.

Table 58:- Number of Bacteria-Carrying Dust Particles per Cubic Foot of Air in a 100 Cu.Ft. Chamber while Various Bodily Activities Undertaken by Subject A. with and without Gowning as in Figure 14.

Activity:-	Standing Motionless			"Operating" (Arm & body movements only)						"Marching" (Swinging arms & raising feet)						Undressing & Dressing.	Brushing Clothes
Gowning:-	No Gown	Surg-ical	Dust-proof	No Gown		Surgical Gowning		Dust-proof Gowning		No Gown		Surgical Gowning		Dust-proof Gowning		No Gown	No Gown
Culture Medium	BA	BA	BA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	BA
Experiment No.	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Control Period																	
0 - 10 min.	2	1	1	1	2	1	1	1	1	1	3	2	1	2	1	1	2
11 - 21 min.	5	1	1	2	1	1	1	1	1	4	3	4	2	2	2	1	3
Activity Period																	
22 - 24 min.	15	10	6	47	105	42	98	5	24	289	447	145	224	16	15	551	490
24.7-26.7min.	11	7	6	84	210	61	153	11	19	906	1148	385	330	33	34	1880	555
27.3-29.3min.	7	8	6	147	242	73	131	9	23	1197	1737	736	392	35	30	2375	817
30 - 32 min.	7	5	4	274	335	119	211	11	27	1063	1804	656	289	41	36	2654	647
Die-away Period																	
33 - 35 min.	7	5	4	134	200	76	136	9	16	615	1356	348	204	33	25	1849	483
37 - 39 min.	12	7	5	93	148	35	104	7	21	445	1096	304	182	31	28	1215	272
42 - 44 min.	7	5	6	61	104	27	72	4	-	345	913	227	129	35	27	686	199
52 - 54 min.	7	5	8	20	29	10	59	4	18	133	521	140	91	22	14	377	51
72 - 74 min.	5	4	5	4	13	5	47	3	9	48	276	69	-	19	10	126	12
92 -102 min.	2	3	5	2	6	2	20	1	10	19	101	40	21	12	6	46	8
152 -162 min.	1	1	2	1	1	1	9	1	3	8	33	18	9	-	3	-	7
212 -222 min.	1	1	2	1	1	1	4	1	-	5	5	5	4	3	3	2	4
272 -282 min.	1	1	1	1	1	1	1	1	3	4	2	5	3	2	2	1	3

See text for explanation of legends. BA: blood agar. CA: chocolate agar. - means no sample.

Table 59:- Number of Bacteria-Carrying Dust Particles per Cubic Foot of Air in a 100 Cu.Ft. Chamber while Various Bodily Activities Undertaken by Subject K. with and without Gowning as in Figure 14.

Activity:-	Standing Motionless			"Operating" (Arm & body movements only)						"Marching" (Swinging arms & raising feet)						Undressing & Dressing	Brushing Clothes
Gowning:-	No Gown	Surg-ical	Dust-proof	No Gown		Surgical Gowning		Dust-proof Gowning		No Gown		Surgical Gowning		Dust-proof Gowning		No Gown	No Gown
Culture Medium	BA	BA	BA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	CA	BA	BA
Experiment No.	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Control Period																	
0 - 10 min.	1	2	1	3	8	2	3	1	3	5	2	-	2	2	4	1	1
11 - 21 min.	1	2	2	3	9	3	4	2	3	2	4	1	2	1	4	2	1
Activity Period																	
22 - 24 min.	8	11	7	35	49	23	27	10	18	337	465	601	206	14	28	673	323
24.7-26.7min.	9	7	7	63	62	26	17	12	12	707	413	730	330	25	33	1214	294
27.3-29.3min.	4	12	7	73	45	36	20	9	9	726	600	855	321	31	31	1401	329
30 - 32 min.	3	7	10	113	78	45	19	11	8	744	632	866	417	26	33	2139	580
Die-away Period																	
33 - 35 min.	9	12	12	58	33	24	17	10	12	458	381	417	229	24	28	1810	505
37 - 39 min.	8	7	8	65	31	18	26	11	16	315	314	306	233	17	28	1152	351
42 - 44 min.	7	8	7	28	16	17	12	10	10	185	-	96	214	13	20	962	168
52 - 54 min.	5	10	9	21	11	12	12	9	8	114	88	56	142	6	21	536	126
72 - 74 min.	5	9	8	10	13	11	9	3	5	34	37	154	68	5	10	247	59
92 -102 min.	3	6	6	9	6	5	3	5	4	14	14	-	37	3	6	87	17
152 -162 min.	1	5	3	7	4	2	2	3	2	5	6	-	9	2	3	31	6
212 -222 min.	1	4	2	5	1	1	2	3	-	2	4	-	3	1	3	10	2
272 -282 min.	2	4	3	6	-	5	2	2	1	2	3	-	4	1	4	3	1

See text for explanation of legends. BA: blood agar. CA: chocolate agar. - means no sample.

Table 60a:- Summary of Results of 28 Experiments in Tables 56-59: Amount of Air Infection with Dust from Skin and Clothing Produced during Various Bodily Activities by Persons Wearing Ordinary Non-sterile Clothes.

Nature of Activity (10 Minutes)	Subject	BACTERIA-CARRYING PARTICLES:- Average Number per Cu.Ft. of Air during the Activity Period	Total Number in 100-Cu.Ft. Box at 1-3 Minutes after Activity.
Standing Motionless	D.	11	900
	W.	7	600
	A.	10	700
	K.	6	900
	Average	9	800
"Operating" Arm & body movements.	D.	170	18,100
	D.	307	25,000
	W.	23	2,500
	W.	37	4,800
	A.	138	13,400
	A.	223	20,000
	K.	71	5,800
	K.	59	3,300
	Average	129	11,600
"Marching" Swinging arms & raising feet	D.	1074	92,800
	D.	1743	113,900
	W.	288	24,700
	W.	285	24,600
	A.	864	61,500
	A.	1284	135,600
	K.	629	45,800
	K.	528	38,100
	Average	837	67,100
Undressing & Dressing	D.	2374	154,500
	W.	1091	108,000
	A.	1865	184,900
	K.	1357	181,000
	Average	1672	157,100
Brushing Clothes	D.	888	83,900
	W.	109	15,600
	A.	627	48,300
	K.	381	50,500
	Average	501	49,500

Table 60b:- Summary of 48 Experiments in Tables 56-59: Influence of Wearing a Sterile Gown over Ordinary Non-sterile Clothes upon Amount of Air Infection with Clothing Dust Produced during Bodily Activity.

Nature of Activity (10 minutes)	Subject	Average Number of Bacterial Particles per Cu.Ft. of Air in the 100-Cu.Ft. Chamber during 10 Minutes of Bodily Activity.		
		No Gown	Surgical Gowning	Dustproof Gowning
"Operating" Arm & body movements.	D.	170	149	22
	D.	307	103	20
	W.	23	17	11
	W.	37	48	9
	A.	138	74	9
	A.	223	148	23
	K.	71	33	11
	K.	59	21	12
	Average	129	74	15
"Marching" Swinging arms & raising feet.	D.	1074	955	33
	D.	1743	752	44
	W.	288	159	34
	W.	285	207	26
	A.	864	481	31
	A.	1284	309	29
	K.	629	763	24
	K.	528	319	31
	Average	837	493	32

(Note:- dress and gowning as in Figure 14.)

Table 61:- Number of Bacteria-Carrying Dust Particles per Cubic Foot of Air in a 100-Cu.Ft. Chamber during 10 Minutes of "Marching" by Subjects D. and M. with and without Gowning as in Figure 16.

Subject	Period	Time in Minutes	Ordinary Nonsterile Clothes without Gowning	Ordinary Clothes with Surgical Gowning	Ordinary Clothes with Incomplete Dustproof Gowning		Ordinary Clothes with Complete Dustproof Gowning	
D.	Control	0 - 10	1	1	1	2	2	0
	Activity	12 - 14	835	511	119	106	56	30
		14.7-16.7	981	680	136	130	72	49
		17.3-19.3	908	446	116	113	84	-
		20 - 22	1049	-	114	-	91	61
	Die-away	23 - 25	523	512	79	93	82	32
	Experiment Number:-		(69)	(70)	(71)	(72)	(73)	(74)
M.	Control	0 - 10	1	0	1	1	7	1
	Activity	12 - 14	92	123	74	69	32	7
		14.7-16.7	194	139	118	98	-	6
		17.3-19.3	214	141	150	122	31	2
		20 - 22	188	242	147	138	34	3
	Die-away	23 - 25	164	162	79	91	22	5
	Experiment Number:-		(75)	(76)	(77)	(78)	(79)	(80)

Note:- Incomplete Dustproof Gowning differed from the standard, complete "Dustproof Gowning" in that the gown did not have a close-fitting collar and did not have canvas "socks" sewn to the ends of the trouser legs: the ordinary (but sterile) cotton socks were worn outside the trouser legs.

Table 62:- Summary of 36 Other Experiments (Nos 81-116): Average Number of Bacteria-Carrying Dust Particles per Cubic Foot of Air in a 100-Cu.Ft. Chamber during 10 Minutes of Bodily Activity by Persons Wearing Sterile Clothes with and without Gowning as in Figure 17; Infection from Skin Only.

Activity	Subject	No Gown	Surgical Gowning	Dustproof Gowning
"Operating"	D.	222	100	14
	W.	13	9	4
	J.	49	40	6
	L.	46	98	20
	Average	83	62	11
"Marching"	D.	266	237	20
	W.	75	70	14
	J.	147	185	79
	L.	314	71	15
	Average	200	141	32
"Running"	D.	1138	334	43
	W.	202	274	40
	J.	830	257	69
	L.	67	27	8
	Average	559	223	40

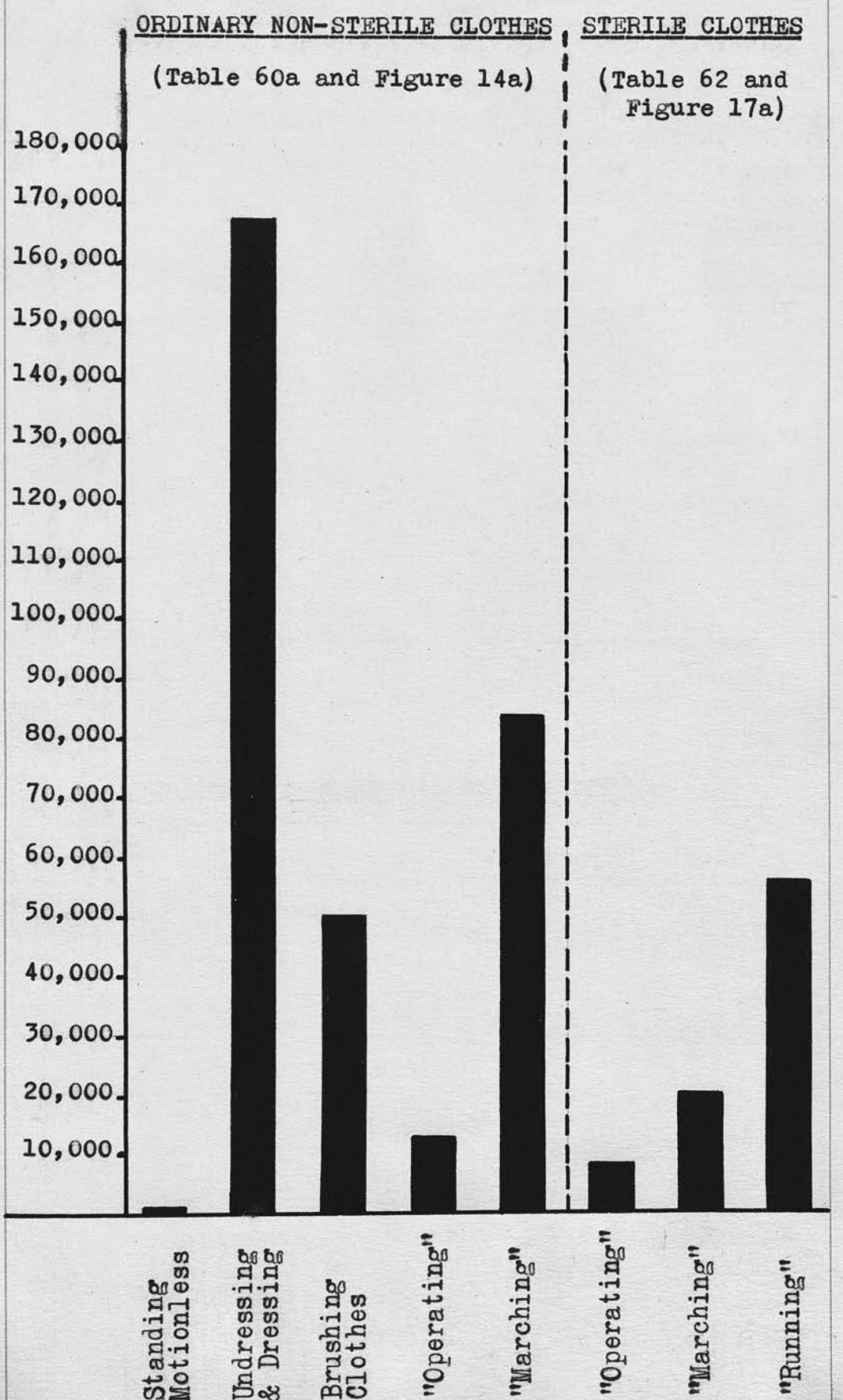
Table 63:- Proportion of Dustborne Bacteria Entering Air from Skin and Clothing which Consists of Alpha Haemolytic Streptococci and Other Alpha Haemolytic Species.

(Subjects wearing ordinary non-sterile clothes undertake various bodily activities. Chocolate agar plates used in slit sampler. From Experiments 1-80, Tables 56-61)

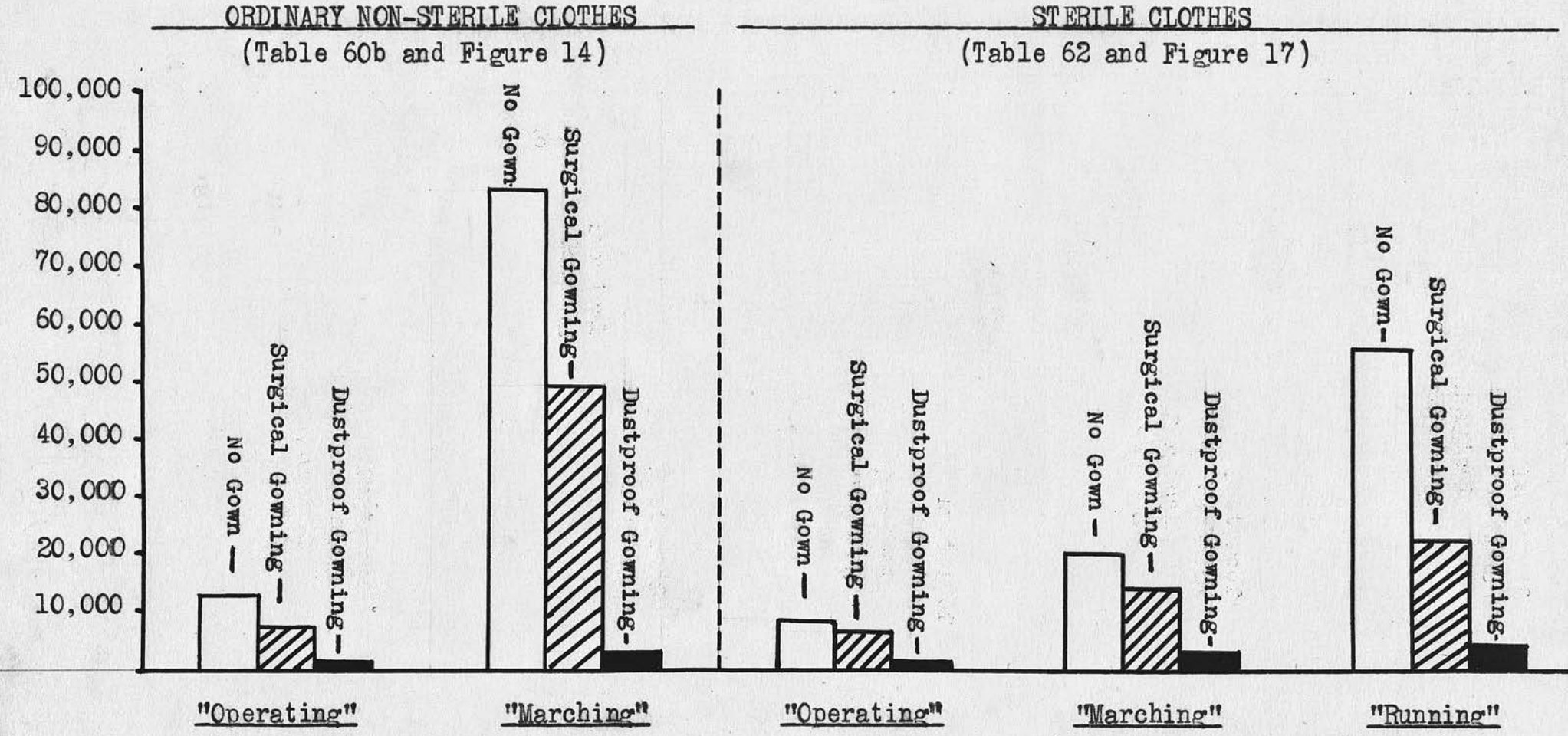
Subject	Number of Experiments	All Colonies	Alpha Haemolytic Colonies	Alpha Streptococcus Colonies	
D.	17	60,217	91	0	(out of 36)
W.	12	14,281	27	0	(out of 20)
A.	6	30,431	44	0	(out of 0)
K.	6	13,510	174	5	(out of 63)
M.	11	11,479	133	19	(out of 98)
S.	9	43,185	117	5	(out of 78)
Total:-	61	173,103	586	29	(out of 295 of the alpha haemolytic colonies filmed)

Of the 173,103 air bacteria, 0.34% were alpha haemolytic bacteria of various species:
0.033% were alpha haemolytic streptococci, and
0.008% were Strept.salivarius (i.e. 5 of 19 alpha streptococci tested)

Graph 8:- Average Results of All Experiments with the Subject Not Gowned: as Average Total Number of Bacteria-Carrying Dust Particles in the 100-Cu.Ft. Chamber during the 10-Minute Period of Occupancy.



Graph 9:- Average Results of Experiments Showing Efficacy of Different Modes of Gowning: as Average Total Number of Bacteria-Carrying Dust Particles in the 100-Cu.Ft. Chamber during the 10-Minute Period of Occupancy and Bodily Activity by the Test Subject.



Graph 10:- Infection of Chamber Air in a Typical Experiment (No.39) Showing Rapid Increase during 10 Minutes of Occupancy and Slight Activity, Followed by a Moderately Rapid Die-away.

(Slight Activity consisted of "Operating" the slit sampler, changing the culture plate four times in 10 minutes. Subject was dressed in his ordinary non-sterile clothes.)

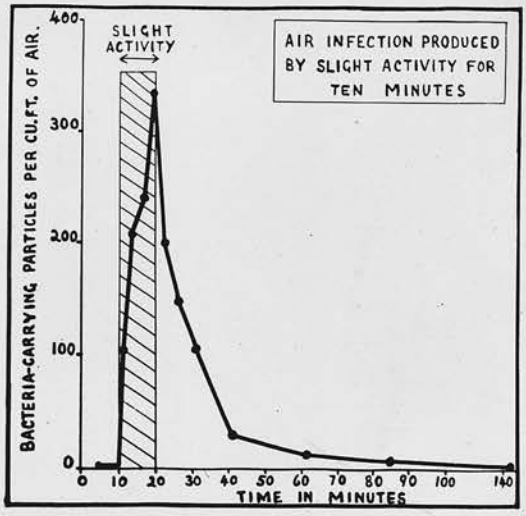


Figure 10:- Photograph of the 100 Cu.Ft. Test Chamber Showing Door, Slit Sampler and Glass Tube Joining Chamber Vent to Sampler Intake.

Figure 13:- Photograph Showing 'Surgical Gowning' (left) and 'Dustproof Gowning' (right) over Ordinary Non-sterile Clothes as in Tests Recorded in Tables 56-60.

(Note:- In this pattern of "Dustproof Gown", the gown collar is tucked inwards, over and down inside the collars of the jacket and shirt of the "Ordinary Clothes" beneath)

Experiments 1-68

Figure 14:- Showing (a) Ordinary Non-sterile Clothes, (b) Sterile Surgical Gown, Cap, Mask, Gloves and Long Rubber Boots over Ordinary Clothes, and (c) Sterile Dustproof Gown over Ordinary Clothes, as in Tests Recorded in Tables 56-60.

(Note:- In this pattern of "Dustproof Gown", a bulky gauze pad attached round the inner surface of the gown collar grips the neck closely at a level above the collars of the "Ordinary Clothes" beneath)

Experiments 1-68

(a)	(b)	(c)
Ordinary Non-sterile Clothes	Surgical Gowning over Ordinary Clothes	Dustproof Gowning over Ordinary Clothes

Figure 15:- Photograph of 'Surgical Gowning' over Ordinary Clothes showing Gaps by which Infected Dust Particles Escape to Air.

Figure 16:- Showing (a) Ordinary Non-sterile Clothes with Sterile Cap, Mask, Gloves and Shoes, (b) Sterile Surgical Gown as well as Cap, Mask, Gloves and Shoes over Ordinary Clothes, and (c) Sterile Dustproof Gown as well as Cap, Mask, Gloves and Shoes over Ordinary Clothes, as in the Tests Recorded in Table 61.

Experiments 69-80

(a) Ordinary Non-sterile Clothes

(b) Surgical Gowning over Ordinary Clothes

(c) Dustproof Gowning over Ordinary Clothes

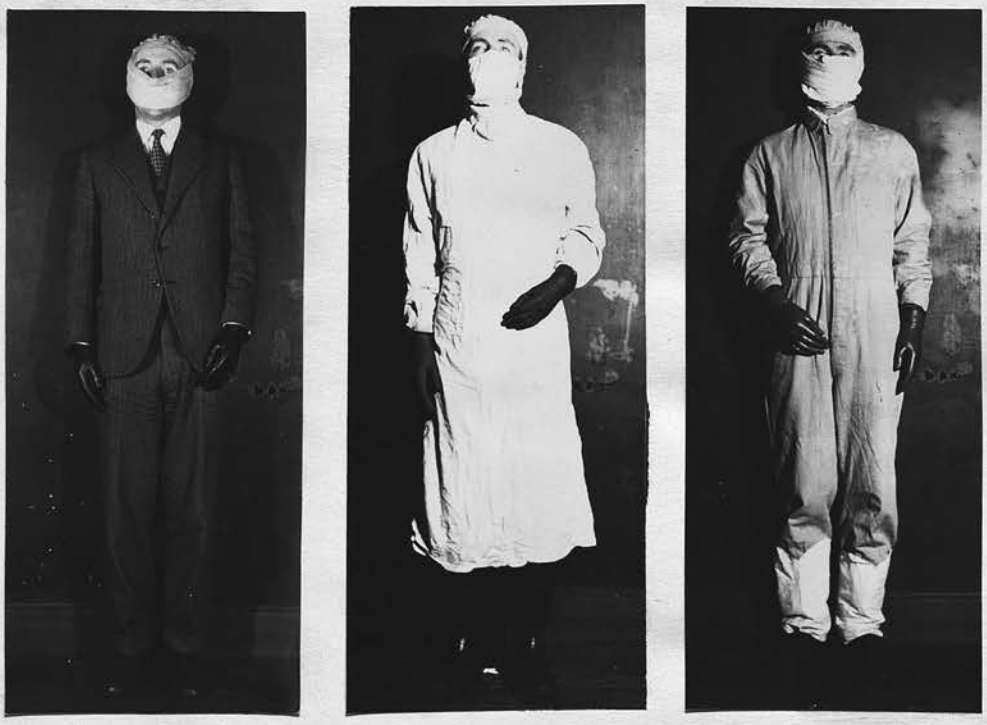


Figure 17:- Photograph Showing (a) Sterile Clothes, (b) Sterile Surgical Gown over Sterile Clothes and (c) Sterile Dustproof Gown over Sterile Clothes, as in Tests Recorded in Table 62.

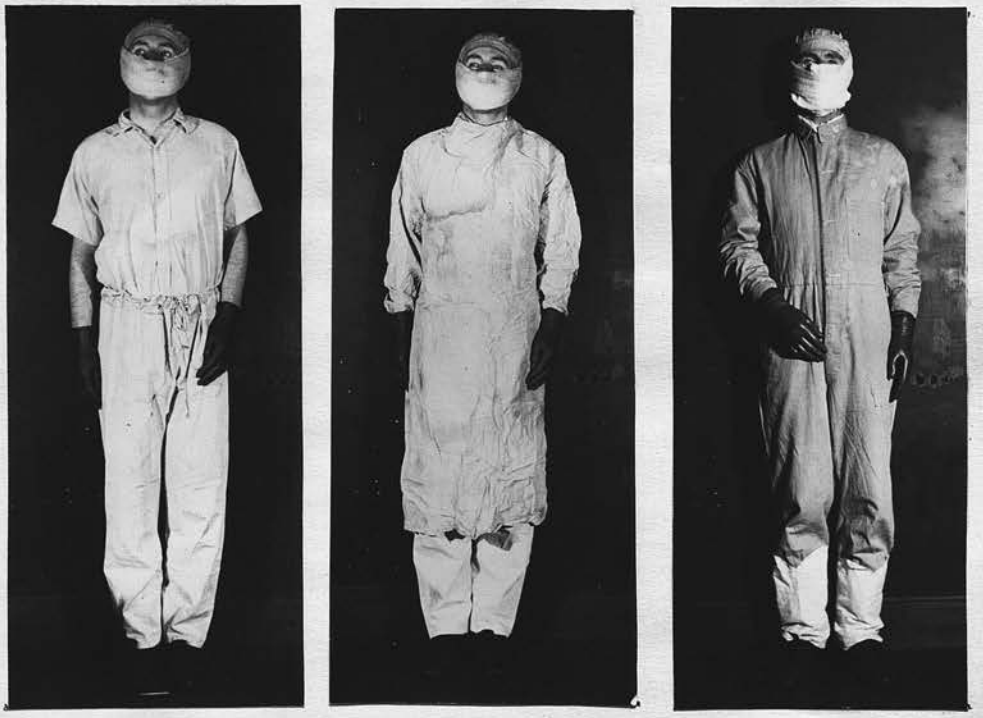
(Note:- Sterile cap, mask, gloves, socks and rubber shoes were worn in all three cases; no other garments were worn under the sterile shirt and trousers seen in the photograph of "Sterile Clothes")

Experiments 81-116

(a) Sterile Clothes

(b) Surgical Gown over Sterile Clothes

(c) Dustproof Gown over Sterile Clothes



Section 16: Role of skin and clothing dust in the transmission of respiratory infections.

The present observations make it clear that very large numbers of bacteria-carrying dust particles are liberated into the air from the skin and clothing as a result of normal bodily activities, and that many of these particles may remain airborne for a considerable time. It is suggested that dust particles from skin and clothing are not inferior to the droplet nuclei of sneezing, coughing and speaking, in supplying the physical means for airborne transmission of infection. The number of bacteria-carrying dust particles given off in the normal activity of daily life must greatly exceed the number of bacteria-carrying droplet nuclei produced by the usual amount of speaking, coughing and sneezing. The observed average rates of 11,600 bacteria-carrying dust particles per 10 minutes of "operating", and 67,100 per 10 minutes of "marching", may be compared with the observed average rates of 13 to 71 bacteria-carrying droplet nuclei per 100 spoken words, 8 to 730 per cough and 39,000 per sneeze (see Table 3; Part 2). Furthermore, bacteria-carrying dust particles from the skin and clothing do not appear to be much coarser and more rapidly sedimenting than bacteria-carrying droplet nuclei, as suggested by Wells, Winslow and Robertson (1946). The finding that 10% of the bacteria-carrying dust particles remained airborne on average for about 35 minutes is to be compared with the finding/

finding that 10% of sneeze-produced bacteria-carrying droplet nuclei remained airborne on average for 40 to 45 minutes (see Tables 22 and 24; Part 2).

Section 17: Role of airborne dust from the skin and clothing in causing operation wound infection.

The pyogenic organisms most often infecting wounds - Staph.aureus and Strept.pyogenes - commonly originate from the respiratory tract of carriers. Dissemination of infection from the respiratory tract via clothing dust to the air, constitutes a means whereby surgical aseptic technique may be breached; infected dust particles may settle from the air on to the operation wound, the surgeon's hands, instruments and dressings. Staph.aureus and Strept.pyogenes have been demonstrated in the air of operating-rooms by Hart (1938), Hart and Schiebel (1939), and MacDonald (1940). Hart attributed frequent "unexplained infections" in clean operation wounds to this aerial infection. Colebrook and Ross (1947) demonstrated infection of the air of a burn dressing-room with Strept.pyogenes which was proved by typing to be derived from a small sore on the surgeon's elbow; apparently the gown worn by the surgeon was not effective in preventing dissemination of infected dust from this site.

The present findings show that a large amount of dustborne bacterial contamination of the air may be caused by ordinary body movement. A person wearing ordinary non-sterile clothes liberated, on average, over 11,600 bacteria-carrying particles per 10 minutes of "operating", and a person wearing sterile clothes liberated, on average, over 8300 bacterial particles per/

per 10 minutes of vigorous "operating".

Furthermore, the present findings show that some of these infected dust particles from the skin and clothing will remain airborne for a time more than sufficient to allow their drifting to the operating-table from all parts of the theatre and from rooms and corridors adjoining.

The loose cotton gown usually worn by surgeons was unable to prevent bacterial contamination of the air from the skin and clothing. Air infection is likely to be produced if surgeons, nurses, anaesthetists, students, or other attendants enter the operating-theatre or its gallery while clad in their ordinary clothes covered only by a loose gown.

Obviously it is desirable that the surgeon and operating-staff should change from their ordinary clothes into special operating-clothes, shirt and trousers, which are sterile. However, a surgeon dressed exclusively in sterile clothes will, by the movements of operating, still infect the air with many bacteria-carrying particles derived from his skin. Wearing of the loose surgical gown over his sterile operating-clothes will not prevent such air infection.

Section 18: Precautions necessary in bacteriological examination of air.

When examining air for its total bacterial content, an observer should take precautions against himself infecting the air, lest the counts obtained should reflect mainly his own activities in manipulating the air sampler and not the natural air contamination of the place examined. The movements involved in operating an air sampler have been proved sufficient to produce heavy bacterial contamination of air, by the present observations for "operating" an imaginary slit sampler.

In view of these findings, it is proposed that a sterile dustproof gown should be worn by any person engaged in taking air samples for bacteriological examination.

Section 19: Summary of Part 3.

(1) The number of bacteria-carrying dust particles liberated from a person's skin and clothing into the air of a 100-cu.ft. chamber as a result of various bodily activities was measured by examining the air with a Bourdillon slit sampler. Large numbers of bacteria-carrying particles were liberated by even slight activity - e.g. about 1000 per minute by a person making movements equivalent to performing a surgical operation or to changing culture plates in a slit sampler. Very large numbers were liberated by more vigorous activities - e.g. about 10,000 per minute by a person "marching" or changing his clothes. The average number of bacteria-carrying particles found in the chamber air at 1 to 3 minutes after 10 minutes of occupation, was 800 after the occupant had been "standing motionless", 11,600 after he had been "operating" an imaginary slit sampler, moving only his arms and body, 67,100 after he had been "marching", swinging his arms and lifting his feet, 157,100 after he had been "undressing and dressing" himself once, and 49,500 after he had been "brushing clothes", giving 40 strokes of a brush to his jacket and trousers.

(2) Observations were made of the rate at which the bacteria-carrying dust particles from skin and clothing disappeared from the air after termination of the dust-raising activity. On average, 10% of the infected/

infected particles remained airborne for 35 minutes.

(3) The respiratory tract commensal bacterium, Strept.viridans, was found on a proportion of the dust particles liberated into the air from the skin and clothing. This proved the probability that pathogenic organisms from the respiratory tract would pass by the same route via skin and clothing dust into the air. A few Strept.viridans were put into the air by 3 out of 6 persons examined in 61 experiments. The culture plates exposed in the 61 experiments bore 173,103 colonies of all kinds; 0.34% of these were alpha haemolytic, but only 0.033% were alpha haemolytic streptococci.

(4) Comparison of numbers disseminated and duration of air-carriage indicated that the dust particles from skin and clothing are not inferior to the droplet nuclei of speaking, coughing and sneezing in supplying the physical means for airborne transmission of infection.

(5) Air contamination with dustborne bacteria from the skin and clothing was reduced only a little - e.g. to about half - when a sterile loose cotton gown of the usual surgical pattern was worn over the ordinary clothing, but was reduced very greatly - e.g. to a tenth or a twentieth - when a sterile dustproof gown was worn over the ordinary clothing.

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Part 4

EXPERIMENTAL INVESTIGATION OF DISSEMINATION BY INFECTED PERSONS OF SPECIFIC PATHOGENIC BACTERIA OF THE RESPIRATORY TRACT, NAMELY, M. TUBERCULOSIS, C. DIPHTHERIAE, STREPT. PYOGENES AND STAPH. AUREUS.

CONTENTS OF PART 4

Section 1: General Introduction.

Studies of the physical mechanisms of droplet spray and dust raising, as reported in Parts 2 and 3, yield much information about the possibility of infection being spread by these vehicles. However, because a number of particles are distributed into the air and remain airborne for some time, it does not follow that airborne infection will necessarily be produced; it is possible that none of the particles carry pathogenic microorganisms.

The role of the different vehicles of infection, large projection droplets, airborne droplet nuclei, airborne dust particles, hands, clothing and fomites, can be assessed only with knowledge of the extent to which each is normally contaminated with pathogenic microorganisms. This knowledge can be gained only by direct bacteriological examination of the vehicles, in relation to a wide selection of infected persons. In the present investigation, such examination has been made in relation to 20 patients with open pulmonary tuberculosis, 50 patients with faucial diphtheria, 50 patients with scarlet fever, 45 "healthy carriers" of Strept.pyogenes and patients with chronic tonsillitis and 2 "healthy nasal carriers" of Staph.aureus. The tuberculosis, diphtheria and scarlet fever patients were inmates of an Infectious Fevers Hospital, the patients with chronic tonsillitis were inmates of an Ear, Nose and Throat Hospital Unit, and the "healthy carriers"/

carriers" were encountered under a variety of circumstances,

The most extensive observations concerned the expulsion of pathogenic bacteria in cough spray and the occurrence of pathogenic bacteria in the secretions of the anterior mouth. Particular attention was paid to this latter, because the observations recorded in Part 2 had shown that the great majority of the droplets produced in speaking, coughing or sneezing, originate from the anterior mouth; it had been concluded that airborne infection by droplet nuclei would be likely only when the secretions of the anterior mouth contained very large numbers of pathogenic microorganisms.

The three experiments described in Section 12 were made in collaboration with Dr. C.A.Green and Dr. S.W. Challinor; the observations recorded in Section 16 were made in collaboration with Dr. A.T.Wallace.

Part 4a

DISSEMINATION OF M. TUBERCULOSIS

Section 2: Experimental methods.

The patients were examined at a considerable interval after their last meal or mouth-wash, and not immediately after a bout of coughing. Each patient was instructed to give 6 forceful coughs. As the coughs were voluntary, they may be regarded as "unnatural". Sometimes, however, the disturbance of the first cough initiated the cough-reflex and some "natural" coughs were included in the series.

The droplets expelled by the series of 6 coughs were collected on a glass microscope slide (3 in. x 1 in.), which was held during the coughing at about 3 inches directly in front of the mouth. The slide was always warmed just before use so as to prevent condensation from the breath which would have obscured the outlines of the droplet marks. The slide was stained by the Ziehl-Neelsen method and then searched systematically for droplet marks under the low power of the microscope. The diameter of each droplet mark was measured with a micrometer eyepiece. Finally, the droplet marks were searched for tubercle bacilli with the (1/12 in.) oil-immersion objective. A record was made of the number of droplets on each slide, the number of droplets containing tubercle bacilli, and the number of tubercle bacilli in each droplet.

Before and after each test of coughing, a swab was taken from the throat (tonsils, faucial pillars and posterior pharyngeal wall) and another swab was taken from/

from the anterior mouth (inner surface of lips, inner and outer surfaces of front teeth and gums, and tip of tongue). The swabs were treated with acid according to the method of Nassau (1941) and each was then rubbed over the surface of two Löwenstein egg-medium slopes. These slopes were incubated at 37 deg.C. for four weeks; they were examined at intervals during this time. The number of M.tuberculosis colonies visible after four weeks was counted and recorded.

Section 3: Observations of M.tuberculosis in cough spray and in secretions of anterior mouth.

Observations were made of the expulsion of tubercle bacilli during 6 voluntary coughs given by each of 20 adult male patients with open pulmonary tuberculosis, whose sputum had recently been shown to contain tubercle bacilli. Table 64 shows for each patient the numbers of droplets and tubercle bacilli which were caught on a microscope slide (3 in. x 1 in.) held at 3 inches in front of the mouth, and also the occurrence of tubercle bacilli, before and after coughing, in the throat and in the anterior mouth.

Droplets containing tubercle bacilli were expelled by 10 out of the 20 tuberculosis patients. In all, a total of 410 droplets were found on the slides exposed to 120 coughs; 36 of these droplets were infected, 19 containing between 1 and 10 tubercle bacilli, 9 containing between 10 and 100, 3 containing between 100 and 1000, 4 containing between 5000 and 20,000, and 1 containing about 40,000. The bacilli were packed so closely in the five most heavily infected droplets that only a rough estimate could be made of their numbers. These 5 heavily infected droplets and 10 other infected droplets were all expelled by one patient (No.151), who must have discharged at least 80,000 tubercle bacilli in the 6 coughs. Much smaller numbers of tubercle bacilli were expelled by the other patients; one of the other patients was found to expel 198 tubercle bacilli/

bacilli (in one droplet), a second to expel 175 (in nine infected droplets), a third to expel 60 (in one droplet), a fourth to expel 13 (in three droplets), a fifth to expel 6 (in three droplets), and the other four patients each to expel either 1 or 2 tubercle bacilli in a single droplet.

The deposit marks of the infected droplets ranged from 30 to 3000 microns in diameter, 9 of the 36 being between 30 and 120 microns (i.e. being derived from spherical droplets between about 10 and 40 microns in diameter). It is probable, therefore, that some of the infected droplets were small enough (i.e. under 100 microns) to remain airborne as droplet nuclei. None of these small droplets, however, contained more than a very few tubercle bacilli (not more than 10). The heavily infected droplets which contained more than 1000 tubercle bacilli, were all of large size; their deposit marks were between 600 and 3000 microns in diameter, so that their original spherical diameters must have been between 200 and 1000 microns.

Figure 12 is a photomicrograph of the most heavily infected of the <u>small</u> droplets of the patient (No.151) whose cough spray contained the largest numbers of tubercle bacilli. The deposit mark of this droplet was just under 120 microns in diameter, so that the original spherical diameter of the droplet must have been about 40 microns. Thus, if it had not been caught on the slide, this droplet would have remained airborne/

airborne as a droplet nucleus. As can be seen from the photomicrograph, the droplet contained 9 tubercle bacilli; thus, it would have become an <u>infected</u> droplet nucleus.

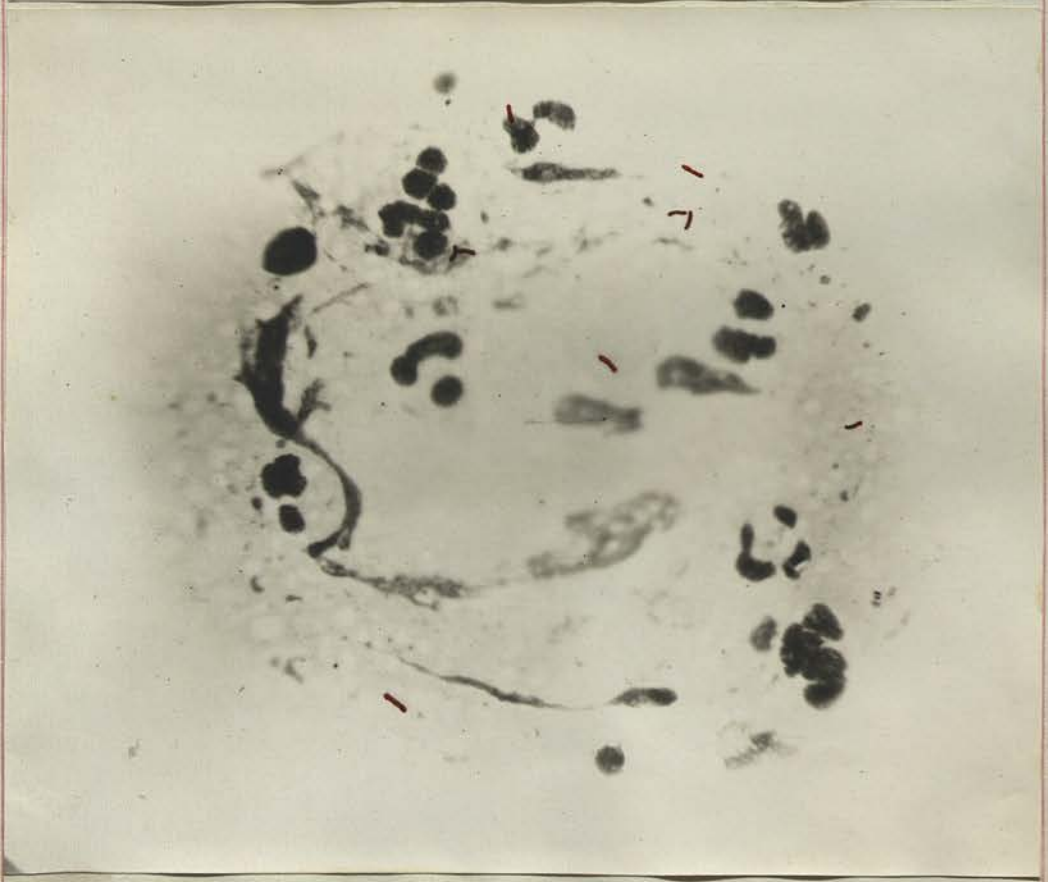
In the examinations made before coughing, tubercle bacilli were found to be present in the secretions of the throat of 15 out of the 20 patients, and in the secretions of the anterior mouth of 10 of the 20 patients. In two other patients, infection of the anterior mouth appeared subsequent to the coughing. The tubercle bacilli were usually numerous in the throat secretions (e.g. a few dozen to a few hundred colonies being obtained on the two slope cultures), and they were usually scanty in the anterior-mouth secretions (e.g. a few to a few dozen colonies being obtained on the two slope cultures). A positive throat culture was obtained from all of the 10 patients who expelled infected droplets, and a positive anterior-mouth culture from 8 of these.

Table 64:- Occurrence of M.tuberculosis in Throat Secretion, Anterior Mouth Secretion and Droplet Spray of 6 Coughs from Each of 20 Persons with Open Pulmonary Tuberculosis.

Patient No.	Number of M.tuberculosis Colonies from Swabs Taken:- before Coughing		after Coughing		Number on 3 in. x 1 in. Cough Slide of:-		
	from THROAT	from ANTERIOR MOUTH	from THROAT	from ANTERIOR MOUTH	NON-INFECTED DROPLETS	INFECTED DROPLETS	TUBERCLE BACILLI
138	700	8	17	2	10	1	1
139	6	0	237	27	0	0	0
140	15	80	136	164	5	1	198
141	0	0	0	0	5	0	0
142	680	32	0	2	17	3	13
143	0	0	0	0	4	0	0
144	56	9	33	0	38	0	0
145	18	0	102	600	25	0	0
146	16	1	0	0	48	1	2
147	94	27	27	46	28	0	0
148	2000	7	600	31	111	9	175
149	199	5	266	2	17	3	6
150	1	0	440	0	0	0	0
151	96	10	600	260	1	15	80,000
152	37	8	215	16	0	1	1
153	1	0	8	0	4	0	0
154	0	0	0	0	12	0	0
155	3	0	0	0	15	1	1
156	0	0	23	0	10	1	60
157	0	0	0	0	24	0	0

Figure 12:- Microphotograph (x 1000) of a Cough Droplet Containing Tubercle Bacilli.

The slide was exposed during coughing in front of the mouth of a patient with pulmonary tuberculosis; it was stained by the Ziehl-Neelsen method. The droplet mark contains polymorph leucocytes and nine tubercle bacilli. The upper photograph is untouched; in the lower, the tubercle bacilli are touched up with red.



Section 5: Discussion and conclusions.

Only the larger droplets are collected on slides exposed in front of the mouth to direct impingement of droplet spray. The size-distribution measurements recorded in Part 2 (see Table 16), indicate that "direct impingement slides" collect only about 30% of all droplets expelled in coughing, mainly the droplets larger than 20 microns in diameter. However, from consideration of the decreasing frequency of tubercle bacilli observed in droplets of decreasing size, it is concluded that the great majority of the infected droplets were larger than 20 microns in diameter. Thus, failure to collect the smaller droplets is not regarded as a serious defect in the method of observation.

An important cause of deficiency in the droplet counts was that the (3 in. x 1 in.) slide which was exposed to the cough spray, would cover only about one-third of the area of cross-section of the spray at 3 inches from the mouth (see Part 2). Thus, the counts which were obtained in this investigation and recorded in Table 64, must represent only about 30% of the infected droplets actually expelled.

Expulsion of tubercle bacilli in cough spray has been observed by several previous investigators (e.g. Laschtschenko, 1899; Heymann, 1901; Ziesché, 1907). The findings of the present investigation are generally similar to those of the previous investigations. Apart from/

from confirming the latter, the present observations are of value in yielding new information about the size of the infected droplets, and about the relationship between expulsion of tubercle bacilli in cough spray and the occurrence of tubercle bacilli in the throat and anterior mouth.

The present findings afford a measure of the probability of infection being spread by two different mechanisms: namely, by projection droplets and by airborne droplet nuclei.

The majority of the infected droplets expelled by coughing, and all the droplets containing more than a few tubercle bacilli, were too large in size to remain airborne as droplet nuclei; they therefore constituted infected projection droplets. In 6 coughs, projection droplets containing large numbers of tubercle bacilli (over 80,000) were expelled by 1 out of 20 patients, and projection droplets containing a few tubercle bacilli by 9 out of 20 patients. It is concluded that the projection droplets of the coughing of patients with open pulmonary tuberculosis commonly transmit a few tubercle bacilli to other persons, and occasionally a large number of tubercle bacilli. This tubercle bacillus content of the projection droplets will be received on to the skin and clothing of persons who are standing within 2 or 3 feet directly in front of the patient during the bout of coughing. Some of these tubercle bacilli may subsequently be transferred from the skin/

skin and clothing of the recipient into his mouth or nose, perhaps by the hands or by redispersion into the air and then inhalation. Probably only a small proportion of the tubercle bacilli would complete this journey to the respiratory tract of the recipient. Cough-spray projection-droplets should not be regarded as a vehicle whereby large numbers of tubercle bacilli may be inoculated into the respiratory tract.

Some of the infected droplets were small enough to remain airborne as infected droplet nuclei; 9 such small infected droplets, with calculated spherical diameters between 10 and 40 microns, were collected from 120 coughs. These small infected droplets contained only a few tubercle bacilli (1 to 10). It is concluded that a person standing near to a tuberculous patient during a bout of coughing, will only rarely inhale an infected droplet nucleus, and that the number of tubercle bacilli acquired in this way will be very small.

A positive throat culture was obtained from all of the 10 patients who expelled infected droplets, and a positive anterior-mouth culture from 8 of these. There was not any evidence to suggest that infected material was atomised in the bronchi and expelled as droplets directly from the bronchi. Formation of infected droplets probably occurred in the throat or in the anterior mouth, these localities having been soiled previously by sputum coughed up from the bronchi.

Part 4b

DISSEMINATION OF C. DIPHTHERIAE

Section 6: Experimental methods.

The patients were examined at a considerable interval subsequent to their last meal or last mouth-wash. Each was instructed to give 6 voluntary coughs; these were in most cases fairly forceful, but rather weak in about a quarter of the cases, mainly among the children.

The droplets expelled by the series of 6 coughs were collected on a 10-sq.in. plate of Hoyle's laked blood tellurite agar; this plate was held at about 3 inches directly in front of the mouth during the coughing.

After their aerobic incubation at 37 deg.C. for 48 hours, the plates were examined with a (x 20) binocular plate microscope. Separate counts were made of the colonies resembling C.diphtheriae and of all other colonies. Some of the C.diphtheriae-like colonies on each plate were subcultured and their fermentation reactions were tested; those which fermented glucose but not sucrose, were recorded as C.diphtheriae.

Before each test of coughing, a swab was taken from the throat (tonsils, faucial pillars and posterior pharyngeal wall) and another from the anterior mouth (inner surface of lips, inner and outer surfaces of front teeth and gums, and tip of tongue). These swabs were plated out on Hoyle's medium; after 48 hours incubation, the C.diphtheriae colonies were identified as just described, and their approximate number noted.

Section 7: Observations of C.diphtheriae in cough spray and in secretions of anterior mouth.

Observations were made of the expulsion of C.diphtheriae during 6 voluntary coughs which were given by each of 50 patients with faucial diphtheria. The patients included adults and children older than 6 years; all the patients yielded C.diphtheriae on the throat swab taken just before the test of coughing. Table 65 shows for each patient the number of droplets containing C.diphtheriae which were caught on the 10-sq.in. Hoyle plate held in front of the mouth, and also the occurrence of C.diphtheriae in the throat and in the anterior mouth.

Droplets containing C.diphtheriae were found to be expelled, in a series of 6 coughs, by 10 out of the 50 patients with faucial diphtheria; only 1 infected droplet was expelled in 5 of these cases, 2 infected droplets in 3 cases, and, respectively, 10 and 27 infected droplets in the other 2 cases. In all, 48 infected droplets were expelled during 300 coughs, that is about 1 infected droplet for every 6 coughs. The infected droplets comprised about 4% of all the droplets (which contained organisms cultivable on the tellurite medium) found to be expelled by the 50 patients. It must be noted that the Hoyle tellurite medium is selective for C.diphtheriae and inhibitory to many of the respiratory tract commensal bacteria. For this reason, the number of colonies other than of C.diphtheriae, probably underestimates greatly the actual/

actual number of droplets caught on the plates.

Diphtheria bacilli were discovered in the anterior-mouth secretions of 12 out of the 50 patients; only very small numbers were present in 10 of these patients and moderate numbers in the 2 others. There was not a complete correlation between the expulsion of infected droplets and the presence of C.diphtheriae in the anterior mouth. Infected droplets were expelled by 6 of the 12 patients with positive anterior-mouth cultures (in all, 43 infected droplets) and by 4 of the 38 patients with negative anterior-mouth cultures (in all, 5 infected droplets).

Table 65:- Number of C.diphtheriae Colonies on Hoyle Agar Plates Inoculated with (1) Throat Secretion, (2) Anterior Mouth Secretion and (3) Droplet Spray of 6 Coughs. from 50 Diphtheria Patients.

Patient No.	Throat	Anterior Mouth	Colonies on Cough Plate C.diphtheriae	Others
88	many	0	0	11
89	many	0	0	33
90	many	few	27	24
91	many	0	0	48
92	many	some	10	68
93	many	0	0	158
94	many	0	0	32
95	many	few	0	22
96	many	few	2	13
97	many	0	0	5
98	many	0	0	5
99	many	0	0	3
100	many	0	1	52
101	few	few	1	89
102	many	0	0	11
103	many	few	0	168
104	many	0	1	14
105	many	0	1	72
106	many	0	0	9
107	many	0	0	10
108	many	0	0	13
109	many	0	0	29
110	many	few	2	11
111	many	0	0	34
112	few	0	0	16
113	few	0	0	3
114	many	0	0	6
115	many	0	0	2
116	many	few	0	5
117	few	0	0	3
118	many	0	2	5
119	many	0	0	3
120	few	0	0	11
121	many	some	0	0
122	few	0	0	0
123	many	0	0	7
124	many	0	0	6
125	many	few	1	3
126	many	0	0	2
127	many	0	0	0
128	many	0	0	2
129	many	0	0	6
130	many	0	0	3
131	few	0	0	4
132	many	0	0	0
133	many	few	0	55
134	many	few	0	13
135	few	0	0	16
136	many	0	0	0
137	many	0	0	1

Section 9: Discussion and conclusions.

The cross-section of cough spray at 3 inches in front of the mouth was found in experiments recorded in Part 2 to be about 10 sq.in.; the area of the Hoyle plate was 10 sq.in., so that the whole extent of the spray would be covered by the plate if its positioning was correct. The droplet size-distribution measurements recorded in Part 2 (see Table 16) showed that the "direct impingement plates" collect only about 30% of all cough droplets, mainly those larger than 20 microns in diameter. However, C.diphtheriae occurred in only a small proportion of the larger, impinging droplets; the proportion of smaller droplets infected must necessarily have been less, in fact insignificantly little. For this reason, failure to collect the smaller droplets is not regarded as a serious defect in the method of observation.

The present findings accord with those of Teague (1913) who collected cough spray and speech spray on Loeffler serum plates and identified C.diphtheriae solely by its morphological appearance in methylene blue smears. Both investigations show that only very small numbers of C.diphtheriae-containing droplets are expelled; in the present investigation, the average number of infected droplets per cough varied among the different patients from 0 to 4.5, the overall average rate being 0.16 per cough.

It is concluded that C.diphtheriae may occasionally be/

be transmitted by projection droplets or by airborne droplet nuclei, although only in very small numbers.

In contrast, large numbers of C.diphtheriae have been found in floor dust and in air contaminated with dust by sweeping (Crosbie and Wright, 1941); comparison with the present findings leads to the conclusion that infection by projection droplets and infection by airborne droplet nuclei are less important than infection by airborne dust.

C.diphtheriae-containing droplets were found to be expelled more frequently and in larger numbers by patients having C.diphtheriae in the anterior mouth than by patients having C.diphtheriae in the throat only. This suggests that the majority of droplets, including infected droplets, originated from the anterior mouth and that only a few originated directly from the throat.

Part 4c

DISSEMINATION OF STREPT.PYOGENES

Section 10: Experimental methods.

The patients and "carriers" were examined at a considerable interval subsequent to their last meal or mouth wash. Each was instructed to give 6 voluntary coughs; in most cases the coughing was vigorous.

The droplets expelled by the series of 6 coughs were collected on a 10-sq.in. plate of blood agar (nutrient agar with 5% horse blood); this plate was held during coughing at about 3 inches directly in front of the mouth.

After aerobic incubation at 37 deg.C. for 24 hours, separate counts were made of the beta haemolytic colonies and of all other colonies. Some of the beta haemolytic colonies on each plate were subcultured into 5% serum broth and tested for production of a soluble haemolysin for sheep red blood corpuscles. Only those strains which produced a soluble haemolysin were counted as Strept.pyogenes.

Before each test of coughing, swabs were taken from the throat (tonsils, faucial pillars and posterior pharyngeal wall) and from the anterior mouth (inner surface of lips, inner and outer surfaces of front teeth and gums, and tip of tongue). These swabs were plated out on blood agar. After 24 hours incubation, the Strept.pyogenes colonies were identified as described above, and their approximate number noted.

Air infection was observed by use of the slit sampler (Bourdillon, Lidwell and Thomas, 1941), which

was/

was operated at an air intake rate of 1 cubic foot per minute and at a slit-plate distance of 3 millimeters. The culture medium used for sampling was blood agar incorporating crystal violet in a concentration of 1 in 1000,000. A few non-selective plates, of ordinary blood agar, were used to count "all bacteria" in the air sampled. The amount of air dust was judged approximately from the number of dust particles seen on the surface of the plates.

Section 11: Observations of Strept.pyogenes in cough spray and in secretions of anterior mouth.

Observations were made of the expulsion of Strept.pyogenes during 6 voluntary coughs which were given by each of 50 patients with scarlet fever and 37 persons with chronic tonsillitis or "healthy throat-carriage". These persons included adults and children older than 6 years; Strept.pyogenes was found on the throat swab taken just before the test of coughing, in the case of each of the 87 persons examined. Tables 66 and 67 show for each person, the number of Strept.pyogenes-containing droplets which were caught on the 10-sq.in. blood agar plate held in front of the mouth, and also the occurrence of Strept.pyogenes in the throat and in the anterior mouth. Table 66 gives the findings for the scarlet fever patients, and Table 67 the findings for the chronic tonsillitis patients and "healthy carriers".

In the series of 6 coughs, droplets containing Strept.pyogenes were expelled by 39 out of the 87 persons with infected throats; 27 persons expelled between 1 and 10 infected droplets, 9 persons expelled between 10 and 100 infected droplets, and 3 persons expelled between 100 and 400 infected droplets. In all, 1109 infected droplets were expelled during 522 coughs: that is, about 2 infected droplets per cough. The greatest numbers of infected droplets were generally expelled by the coughs which were most forceful and which produced most droplet spray. Of

all/

all the droplets expelled by the 87 persons, those containing Strept.pyogenes comprised only 10%. The proportion of droplets infected, varied greatly from case to case, between 0% and 80%.

Strept.pyogenes was found in the anterior-mouth secretions of 13 out of the 87 persons examined. The numbers of Strept.pyogenes found in the anterior mouth were generally small; they were very large in only 2 persons, and comparable with the numbers in the throat secretions in only 4 persons. There was not any apparent correlation between the expulsion of infected droplets and the presence of Strept.pyogenes in the anterior mouth. Infected droplets were expelled by 5 of the 13 persons with positive anterior-mouth cultures (in all, 63 infected droplets and 720 non-infected droplets), and by 34 of the 74 patients with negative anterior-mouth cultures (in all, 1046 infected droplets and 7500 non-infected droplets).

Section 12: Observations of Strept.pyogenes air infection by droplet spray and by dust raising.

Three experiments were made to determine the amount of air infection which could be produced by three different mechanisms: (1) by the droplet nuclei expelled by "throat carriers" in speaking, coughing, singing and laughing, (2) by the raising of dust from the jackets, towels and handkerchiefs of "throat carriers", and (3) by the raising of dust from the floor and bedding of a room normally occupied by "throat carriers". These experiments were carried out in the training institution described in Part 5, during an epidemic of Strept.pyogenes throat infection. The "carriers" used in the experiments were inmates of the institution.

Experiment (i): Air infection with droplet nuclei. Slit sampler observations were made of the bacterial content of the air in the immediate vicinity of four "throat carriers" of Strept.pyogenes, who, while playing a game of cards, talked, laughed and coughed frequently and vigorously. The game was played in the middle of a 16,000 cu.ft. dormitory. Apart from the four "carriers" and the observer, there were not any other persons present in the dormitory during the experiment; the dormitory had been unoccupied for 90 minutes preceding the experiment, a time more than sufficient for disappearance of any pre-existing air infection. The "carriers" were grouped around the slit sampler, being respectively 1½, 2, 4 and 4 feet

from/

from it, and facing either half or fully towards it. To minimise dust-raising, movement was restricted to the requirements of the game. The game lasted for 100 minutes.

Air samples (each of 12 cu.ft.) were taken at intervals before entry of the "carriers", then during the game when the "carriers" were present, and finally during a period after departure of the "carriers". Table 68 shows the level of Strept.pyogenes air infection at different times.

Few Strept.pyogenes particles were present in the air during the periods before and after the game, when the dormitory was unoccupied (on average, respectively 0.03 and 0.13 infected particles per cu.ft.). During the 100-minute game, while the "carriers" were talking, coughing, singing and laughing, a larger number of Strept.pyogenes particles were present in the air; a total of 84 cu.ft. of air sampled was found to contain 62 Strept.pyogenes particles, or 0.74 per cu.ft.. As this air infection must have been concentrated in the part of the 16,000 cu.ft. dormitory near to the "carriers", the output per "carrier" was probably less than 3000 infected particles in the 100 minutes.

Thus, a great amount of coughing, speaking, singing and laughing by four infected persons produced only a slight increase in the level of air infection. The times at which increased air infection was observed, did not correspond to the times of maximum coughing and talking/

talking; this suggests that the air infection increase was caused by some mechanism other than droplet spray.

Experiment (ii): Air infection with dust from jackets, towels and handkerchiefs. Slit sampler observations were made of the bacterial content of the air in a small room of 1960 cu.ft. capacity (14 ft. x 14 ft. x 10 ft.) during the shaking of their jackets, towels and handkerchiefs by four "throat carriers" of Strept.pyogenes. The conditions of the experiment were arranged so that air infection could not arise except from the skin, clothing and belongings of the "carriers". The room had, on the previous day, been emptied of furniture and hangings, and had been treated thoroughly with formalin. After the floor had been scrubbed with antiseptic, the room was left unoccupied until the time of the experiment. Because of these precautions, the production of air infection by the raising of room dust was rendered highly improbable. To prevent the expulsion of droplet spray, the "carriers" were masked while present in the room.

"Dust raising" was effected as follows: (1) the "carriers" marched continually round the room for 6 minutes, thus agitating their clothes gently; and (2) the "carriers", during 16 minutes, shook in succession their jackets, towels and handkerchiefs towards the slit sampler in the centre of the room.

Air samples, each of 6 cu.ft., were taken before, during/

during and after each "dust raising". Table 69 shows the level of Strept.pyogenes air infection at different times.

Only a few Strept.pyogenes particles were present in the air before entry of the "carriers", on average 0.4 per cu.ft.. These streptococci probably were derived from the clothing of the two observers who had earlier been in contact with other "carriers". A small but definite increase in the level of Strept.pyogenes air infection resulted from the "carriers" walking around the room, presumably due to liberation of dust from their skin and clothing; the air infection was increased from 0.4 to 1.8 Strept.-pyogenes particles per cu.ft., representing an addition to the 1960 cu.ft. room of about 700 Strept.pyogenes particles by each "carrier". When the four "carriers" shook their jackets, towels and handkerchiefs, there was a very great increase of air infection with Strept.pyogenes, from 1.8 to about 200 infected particles per cu.ft., representing an addition to the air of the whole room of about 100,000 Strept.pyogenes particles by each "carrier". High "air dust" measurements were obtained at the time of the high air infection. After cessation of the "dust raising" and departure of the "carriers", large numbers of Strept.pyogenes particles remained airborne for at least 15 minutes.

Experiment (iii):/

Experiment (iii): Air infection with dust from floor and bedding. Slit sampler observations were made of the bacterial content of the air during sweeping and the shaking of bedclothes, in a 16,000 cu.ft. dormitory usually occupied by 18 young men including 6 "throat carriers" of Strept.pyogenes. The "dust raising" was carried out by 6 young men who were not carrying Strept.pyogenes. These young men, and also the two observers, were masked to prevent expulsion of droplet spray. No other persons were present in the dormitory during the experiment or during the preceding hour.

Dust was raised in three diferent ways: (1) the youths marched continuously up and down the dormitory during 13 minutes; (2) the youths swept the floor towards the slit sampler during 17½ minutes; and (3) the youths shook, close to the slit sampler, various blankets, towels and pillows belonging to the usual occupants of the dormitory, during 18 minutes.

The slit sampler was situated centrally in the dormitory. Air samples were taken before, during and after each "dust raising". Table 70 shows the level of Strept.pyogenes air infection at different times.

Strept.pyogenes was not found in the air during the preliminary period before the activities were begun. In marching up and down the dormitory, presumably by raising dust from the floor, the youths produced a small amount of air infection with Strept.pyogenes; on average, 0.1 infected particles were/

were present per cu.ft. of air, corresponding to 1600 infected particles in the whole dormitory. Greater air infection was produced by sweeping of the floor, namely, 4.9 Strept.pyogenes particles per cu.ft., and by shaking of the pillows, blankets and towels, namely 4.7 Strept.pyogenes particles per cu.ft.. As these latter high air infection levels must have been localised near the sampler, they correspond to an addition of less than 80,000 Strept.pyogenes particles to the whole dormitory.

Total colony counts on two ordinary blood agar plates which were exposed in the slit sampler in the middle of the sweeping and of the bedclothes shaking, showed that at these times there were about 2000 bacteria-carrying particles per cu.ft. of air. Thus, Strept.pyogenes particles comprised only about 0.25% of all the bacteria-carrying dust particles entering the air from the floor and bedding.

High "air dust" measurements were obtained at the times when the air infection was large.

Table 66:- Number of Strept.pyogenes Colonies on Blood Agar Plates Inoculated with (1) Throat Secretion, (2) Anterior Mouth Secretion and (3) Droplet Spray of 6 Coughs, from 50 Persons with Scarlet Fever.

Patient No.	Throat	Anterior Mouth	Colonies on Cough Plate	
			Strept.pyogenes.	Others
1	many	0	102	30
2	many	0	0	3
3	many	0	0	125
4	many	0	5	680
5	many	0	0	42
6	many	0	3	2400
7	few	0	3	220
8	few	0	4	125
9	many	0	0	7
10	few	0	0	2
11	many	few	15	10
12	many	0	47	23
13	many	some	0	2
14	many	0	2	4
15	many	0	0	0
16	many	0	0	0
17	many	few	0	0
18	many	0	1	3
19	many	0	0	6
20	few	0	0	5
21	many	0	0	40
22	many	0	0	12
23	many	0	380	440
24	few	0	0	0
25	many	some	19	17
26	few	0	6	31
27	many	many	18	12
28	many	0	0	1
29	many	few	0	0
30	many	0	1	31
31	many	0	35	485
32	few	0	0	24
33	few	0	0	2
34	few	0	5	20
35	few	0	1	31
36	many	0	28	7
37	many	some	0	7
38	few	0	0	9
39	many	0	0	34
40	many	0	5	16
41	many	0	0	17
42	few	0	0	56
43	few	0	0	130
44	few	0	0	15
45	many	0	0	520
46	few	0	0	3
47	many	0	2	8
48	few	0	17	163
49	many	0	3	19
50	few	0	1	1

Table 67:- Number of Strept.pyogenes Colonies on Blood Agar Plates Inoculated with (1) Throat Secretion, (2) Anterior Mouth Secretion and (3) Droplet Spray of 6 Coughs, from 37 Healthy Carriers and Persons with Chronic Tonsillitis.

Patient No.	Throat	Anterior Mouth	Colonies on Cough Plate	
			Strept.pyogenes.	Others
51	many	few	6	580
52	many	0	20	72
53	many	0	10	292
54	few	0	2	282
55	many	few	0	337
56	many	0	331	210
57	few	0	4	566
58	many	0	1	4
59	few	0	0	40
60	few	0	0	158
61	few	0	0	17
62	many	0	0	360
63	few	0	0	27
64	many	0	2	353
65	many	0	14	186
66	many	some	0	20
67	many	0	2	620
68	many	0	0	53
69	many	some	0	2
70	few	0	0	0
71	many	0	0	3
72	many	0	0	9
73	few	0	1	4
74	many	0	1	27
75	many	few	0	145
76	few	0	0	4
77	few	0	0	13
78	many	many	5	94
79	many	0	0	7
80	many	0	0	14
81	many	0	0	6
82	many	0	1	13
83	few	0	0	9
84	many	0	4	33
85	many	0	0	19
86	many	0	0	30
87	many	0	2	83

Table 68:- Experiment Showing Slight Extent of Droplet Nucleus Air Infection: Number of Strept.pyogenes-Carrying Particles per Cubic Foot of Air in Immediate Neighbourhood of Four Throat Carriers Coughing, Speaking, Singing and Laughing while Seated together at Game of Cards in Large Room.

Time of Observation	Circumstances	Strept.pyogenes per Cubic Foot
1.50 -56 p.m.	Room unoccupied except for Observer.	0.0
2.20 -32 p.m.		0.0
2.35 -47 p.m.		0.1
2.53 -05 p.m.	Four Throat Carriers present: play cards quietly with a little talking and coughing, but from 3.36 to 4.02 p.m. play noisily with much loud talking, laughing, singing and coughing.	0.0
3.07 -19 p.m.		0.0
3.21 -33 p.m.		0.8
3.36 -48 p.m.		0.0
3.50 -02 p.m.		0.1
4.04 -16 p.m.		0.0
4.18 -30 p.m.		4.7
4.32 -44 p.m.	Room unoccupied except for Observer.	0.1
4.45 -57 p.m.		0.3
5.00 -12 p.m.		0.0

Table 69:- Experiment Showing the Heavy Dustborne Air Infection, Expressed as Number of Strept.pyogenes-Carrying Particles per Cubic Foot of Air, Produced by Four Throat Carriers Shaking their Jackets, Handkerchiefs and Towels in a Small Room of 1960 Cu.Ft. Capacity.

Time of Observation	Circumstances	Strept.pyogenes per Cubic Foot	Air Dust
5.26 -32 p.m.	Room unoccupied except for two Observers.	0.3	1
5.33 -39 p.m.		0.5	1
5.40 -46 p.m.	Four Carriers enter and march continuously round the sampler.	1.8	1
5.50 -56 p.m.	Four Carriers shake jackets, towels and handkerchiefs.	150 (approx.)	100
6.00 -06 p.m.		200 (approx.)	100
6.06½-12½ p.m.	Room unoccupied except for two Observers.	250 (approx.)	25
6.13 -19 p.m.		150 (approx.)	10
6.20 -26 p.m.		100 (approx.)	5

Table 70:- Experiment Showing the Moderate Extent of Dustborne Air Infection, as Number of Strept.pyogenes-Carrying Particles per Cubic Foot of Air, Produced by Walking on the Floor, by Sweeping the Floor and by Shaking the Bedding of a 16,000 Cu.Ft. Dormitory Occupied by Throat Carriers.

(Carriers were not present in the dormitory during the experiment).

Time of Observation	Circumstances	Strept.pyogenes Per Cu.Ft.	All Organisms Per Cu.Ft.	Air Dust
1.55 -01 p.m. 2.04 -10 p.m. 2.17 -23 p.m.	Dormitory unoccupied except for two Observers.	0.0 0.0 0.0	- - -	1 1 1
2.29 -35 p.m. 2.36 -42 p.m.	Six Non-Carriers march continuously up and down dormitory.	0.2 0.0	- -	1 1
2.50 -56 p.m.	Six Non-Carriers rest at end of dormitory.	0.0	-	1
3.00 -06 p.m. 3.08 -14 p.m. 3.16 -17½ p.m.	Six Non-Carriers sweep floor vigorously towards air sampler using sterile brushes.	4.8 5.0 -	- - 2000 (approx.)	100 100 -
3.25 -31 p.m. 3.35 -36½ p.m.	Six Non-Carriers rest at end of dormitory.	0.0 0.0	- 65	10 10
3.40 -46 p.m. 3.48 -49½ p.m. 3.52 -58 p.m.	Six Non-Carriers shake near air sampler, the blankets, pillows and towels belonging to Carriers.	7.5 - 1.8	- 2000 (approx.) -	100 100 100
4.02 -05 p.m.	Dormitory unoccupied except for two Observers.	0.7	130	10

Section 14: Discussion and conclusions.

The observations of the expulsion of Strept.pyogenes in cough spray were subject to the limitations of the impingement method of collecting droplets; the plates collect only about 30% of the cough droplets, mainly those larger than 20 microns. However, Strept.pyogenes was contained in only 10% of the droplets collected (i.e. of the large droplets); in proportion to their lesser volume, only a much smaller percentage of the droplets escaping collection can have contained Strept.pyogenes. For this reason, the findings obtained, and recorded in Tables 66 and 67, are assumed to represent substantially the extent of the cough-spray infection.

The present findings are generally similar to the findings of Bloomfield and Felty (1924), Colebrook (1933), Paine (1935) and Hare (1940), who examined only a few infected persons.

The investigation showed that droplet-spray dissemination of Strept.pyogenes was very limited. Only a minority of infected persons, 39 out of 87, expelled any Strept.pyogenes in a series of 6 coughs. The number of Strept.pyogenes-containing droplets expelled, was small, varying among the different persons from 0 to 63 per cough, and being on average about 2 per cough.

It is concluded that transmission of Strept.pyogenes by projection droplets is possible, and perhaps also/

also transmission by airborne droplet nuclei, but that such cough spray infection will be infrequent and that only small numbers of Strept.pyogenes will be passed to the recipients. Certainly, the number of infected droplets found to be expelled in coughing was small as compared with the number of infected dust particles which were found to be raised from floor, bedding and clothing.

The finding that Strept.pyogenes-containing droplets were expelled as frequently by persons with negative anterior-mouth cultures as by persons with positive anterior-mouth cultures, suggests that at least some of the cough droplets, including many infected droplets, were expelled directly from the throat. However, it may have been that the first one or two of the series of six coughs caused movement of infected exudate from the throat to the anterior mouth, and that the later coughs of the series atomised this infected exudate from the anterior mouth.

The three experiments designed to test the different air infecting mechanisms showed that very little air contamination with Strept.pyogenes was produced by the droplet-spray nuclei of "carriers" coughing, speaking, singing and laughing, that a very great air contamination with Strept.pyogenes was produced by the dust liberated on the shaking of jackets, handkerchiefs and towels belonging to "carriers", and that a moderately great air contamination/

ion with Strept.pyogenes was produced by dust raised on sweeping of the floor and shaking of the bedding of a dormitory normally occupied by "carriers".

It is concluded from these observations that airborne infected dust particles constitute a more important vehicle of infection than the projection droplets or airborne nuclei of the respiratory spray of infected persons.

Part 4d

DISSEMINATION OF STAPH. AUREUS

Section 15: Experimental methods.

A prolonged and intensive study was made of two healthy "nasal carriers" of Staph.aureus; both these were male adults. Each was examined repeatedly, on different days throughout the period from May 6th to June 30th, 1948. A variety of bacteriological investigations were made on each day.

The culture medium used for all specimens was that devised by Ludlam (1949) to be selective for Staph.aureus; this is an alkaline nutrient agar containing mannitol, lithium chloride and tellurite (1% Evans peptone, 1% "Lab-Lemco" meat extract, 1% mannitol, 0.5% lithium chloride, 0.5% dipotassium hydrogen phosphate and 2% agar; adjusted to pH 9.3 prior to sterilisation; 0.005% potassium tellurite added just before pouring of the plates). This medium grows all strains of coagulase-positive Staph.aureus as large (2-5 mm. diam.) shiny colonies which are opaque and black-grey uniformly from centre to periphery. The total number of air bacteria capable of growing and forming colonies on Ludlam's medium is not so very much less than the number forming colonies on a non-selective medium such as blood agar; it is usually about 30% to 50% of the latter. However, on Ludlam's medium the majority of these other air bacteria, including Staph.albus, form only very small colonies. The large Staph.aureus colonies are thus easily recognised on Ludlam's medium even when the plate bears numerous colonies /

colonies of all kinds. Some specimens were cultured on blood agar or heated-blood agar as well as on Ludlam's medium.

Swabs were streaked out on the culture plates in the ordinary way; special methods of collecting specimens on to culture plates are described below.

The inoculated culture plates were incubated aerobically at 37 deg.C. for 48 hours. The colonies resembling Staph.aureus were counted and were subcultured on to nutrient agar slopes. The coagulase test was performed on these subcultures. Only those strains which were found to be coagulase-positive were recorded as Staph.aureus. When a very large number of Staph.aureus colonies were present on a culture plate, only about 5 were subcultured and coagulase-tested (however many, all of the Staph.aureus colonies on the air sample plates were tested).

The bacteriological examinations were made as follows:-

(1) Anterior Nares: A sterile cotton-wool swab was moistened with sterile broth and rubbed firmly over the inner surfaces of both nostrils.

(2) Throat: A sterile dry swab was rubbed firmly over the tonsils, faucial pillars and posterior pharyngeal wall; it was not allowed to touch the anterior parts of the mouth.

(3) Anterior Mouth: Usually the examination was made by swabbing; a sterile dry swab was rubbed thoroughly over/

over the inner surfaces of the lips, the inner and outer surfaces of the front teeth and gums, and the tip of the tongue. Sometimes a viable Staph.aureus count was made on saliva from the front of the mouth, which was expectorated into a sterile bottle with care to avoid the hawking forward of secretion from the throat; plate cultures were made of 0.05 ml. amounts of various ten-fold dilutions of this saliva, colony counts were made and the number of viable Staph.aureus per milliliter of the undiluted saliva was calculated.

(4) Lips: A sterile moistened swab was rubbed firmly over the outer surfaces of the lips.

(5) Chin: A sterile moistened swab was rubbed firmly over the skin of the chin.

(6) Chest: A sterile moistened swab was rubbed firmly over about 10 sq.in. of the skin of the chest.

(7) Leg: A sterile moistened swab was rubbed firmly up and down the skin of the shin and calf.

(8) Forearm: A sterile moistened swab was rubbed firmly over the skin of the front and back of the forearm.

(9) Hands: All parts of the fingers, palm, ball of thumb, knuckles and back of hand, in succession, were pressed firmly on the agar surface of a culture plate. Carrier D. pressed both hands on the one culture plate; Carrier S. pressed the right and left hands on different culture plates.

(10) Jacket Front: The observer picked up a culture plate, removed the lid, gripped the plate with the tips of/

of the five fingers of his right hand pressing the rim, and rubbed the plate backwards and forwards over all parts of the outer surface of the front of the carrier's jacket, including the lapels but excluding the sleeves. The surface of the culture medium was held facing the jacket cloth, but rarely came in contact with it. The rim of the plate was scraped firmly over the cloth surface, disturbing dust particles many of which adhered to the medium. "Scraping" was continued until the whole of the medium surface was seen to be lightly powdered with dust.

(11) Jacket Sleeves: A culture plate was scraped in the above manner over the back and front of both sleeves below the level of the elbows.

(12) Shirt Front: A culture plate was scraped over the outer surface of the shirt front in the region of the chest.

(13) Trouser Legs: A culture plate was scraped over the outer surfaces of both trouser legs below the level of the knees.

(14) Handkerchief: The carrier's handkerchief, after it had been in normal use for a few days, was placed in a sterile jar containing 100 ml. of sterile broth. It was left to soak in the broth during 30 minutes, being stirred at intervals with sterile forceps. Finally it was removed from the jar and squeezed with the forceps so that most of the broth drained back into the jar. Plate cultures were made with measured amounts of this broth/

broth eluate and of ten-fold dilutions of it. Colony counts were made and the total number of viable Staph.aureus recovered from the handkerchief was calculated.

(15) Sneeze (First): The droplet spray produced by a sneeze was caught on a 10-sq.in. culture plate held at 3 inches directly in front of the mouth at the moment of sneezing. Carrier D. induced a natural sneeze by tickling his nasal mucosa with a cotton-wool swab. Carrier S. gave a simulated sneeze by forming explosively the sound "ttsch".

(16) Sneeze (Second): Another culture plate was used to collect the spray of a second sneeze given as soon as possible after the first.

(17) 12 Coughs: The carrier gave 12 forceful voluntary coughs. The spray was caught on a 10-sq.in. culture plate held at 3 inches in front of the mouth during the bout of coughing.

(18) Droplet Nucleus Air Infection by Sneezing: The observations of droplet nucleus air infection were made in the 100-cu.ft. test chamber described in Part 3 (Figure 10). The carrier entered quietly, remained in the chamber just long enough to complete his sneezing, and then left quietly, shutting the door. Carrier D. gave two natural sneezes induced by tickling his nasal mucosa with a small cotton-wool swab. Carrier S. gave four simulated sneezes by forming explosively the sound "ttsch". The sneezes were given in quick succession and/

and were directed towards a wall of the chamber at a distance of 3 feet. Air infection with dust from the carrier's skin and clothing was prevented by the carrier avoiding unnecessary movement and wearing a sterile dustproof gown (see Part 3). Air infection with dust from the floor and walls was prevented by treatment of these with spindle oil. With these precautions, it seemed justifiable to assume that any air infection which was observed was due to the sneezing. Air samples were taken by three instruments: a slit sampler (Bourdillon, Lidwell and Thomas, 1941) with an air intake rate of 1 cu.ft. per minute, a sieve sampler (modified from design of Du Buy and Crisp, 1944; see Part 6) with an air intake rate of 19 cu.ft. per minute, and a second sieve sampler with an air intake rate of 23 cu.ft. per minute. The slit sampler withdrew air from the chamber by a vent through the chamber wall at 3 feet above the floor; this vent was screened from above to prevent infected particles falling directly into it. The sieve samplers were operated within the chamber, discharging their exhaust air into the chamber; the air intake openings of these samplers were 1 foot above the floor and were screened from above to prevent particles falling directly into them. The plan of the experiments is shown in Tables 73 and 74. An initial control sample of 10 cu.ft. of air was taken by the slit sampler during the 10 minutes before the carrier entered the chamber; this sample was required for proof that/

that Staph.aureus was not present in the air before the sneezing. At the moment of the first sneeze, the first sieve sampler was started; it was allowed to run for 1 minute, during which time the other sneezes were given; this sample would contain both the large droplets which were in the course of falling to the floor and the small droplets which would remain airborne as droplet nuclei. Taking of the other samples was not started until 1 minute after the last sneeze, so that all the large droplets would have first fallen to the floor and only droplet nuclei would remain in the air. At the end of this minute, two samplers were put into operation simultaneously, the second sieve sampler to take 46 cu.ft. of air in 2 minutes and the slit sampler to take 10 cu.ft. of air in 10 minutes; any Staph.aureus recovered in these samples almost certainly must have been carried in droplet nuclei produced by the sneezing. Thus, during and within a few minutes after the sneezing, 75 cu.ft. of air was examined, three-quarters of the total air content of the test chamber.

(19) Dustborne Air Infection from Skin and Clothing: The observations of dustborne air infection were made in the 100-cu.ft. test chamber; the air was sampled by the slit sampler at the rate of 1 cu.ft. per minute on to plates of Ludlam's medium and plates of heated-blood agar. The carrier wearing his ordinary clothes infected the air by "marching", that is walking without progression/

progression, raising the feet and swinging the arms. Air infection by droplet spray was prevented by the carrier wearing a muslin-cellophane mask over his nose and mouth, and refraining from speech. Air infection by raising of dust from the floor and walls of the chamber was prevented by treatment of these surfaces with spindle oil. With these precautions, it seemed justifiable to assume that any air infection which was observed was due to dust-raising from the skin and clothing. The plan of the experiments was as shown in Tables 71 and 72. An initial control sample of 10 cu.ft. of air was taken on to a Ludlam plate during the 10 minutes before the carrier entered the chamber ("Control Period"); this sample was required to prove the absence of Staph.aureus from the air prior to dust-raising. During the 10-minute "Activity Period", when the carrier was marching in the chamber, three 2-cu.ft. air samples were taken on to Ludlam plates and alternately with these, three ½-cu.ft. samples were taken on to heated-blood agar plates. During the 30-minute "Die-away Period", after cessation of "marching" and vacation of the chamber, three 2-cu.ft. air samples and one 10-cu.ft. sample were taken on to Ludlam plates and, alternately with these, four ½-cu.ft. samples were taken on to heated blood agar plates.

Section 16: Observations of the dissemination of Staph.aureus on hands, clothing, handkerchief, sneeze spray, cough spray, airborne droplet nuclei and airborne dust particles.

During the 8 weeks between 6th May and 30th June 1948, Carrier D. was examined on 22 days and Carrier S. on 11 days. The observations are recorded in Tables 75 and 76.

At every examination both carriers gave profuse growths of Staph.aureus from the anterior nares. The anterior nares appeared to be the main habitat of the staphylococcus, since other parts of the skin and respiratory tract did not yield it in such large numbers or on all occasions. Among the localities sometimes bearing Staph.aureus were the throat, anterior mouth, lips, chin, chest, forearm and hands. It seemed possible that the presence of Staph.aureus on these parts was not due to its growth on them but to its transference from the anterior nares by the hands.

In both carriers the throat usually did not bear any Staph.aureus, but sometimes it bore a few. The anterior mouth of one carrier (S) contained Staph.aureus only rarely and in small numbers (e.g. 10 per ml. on the occasion when a plate count was made); the anterior mouth of the other carrier (D) contained Staph.aureus frequently and sometimes in fairly large numbers (190,000, 5000, 40 and 30 per ml. on four occasions when plate counts were made).

As regards the skin surfaces, the chin and the hands/

hands yielded some Staph.aureus on most occasions; the lips, chest and forearm yielded Staph.aureus on only a few occasions; the leg never yielded Staph.aureus. Great interest attaches to the numbers of Staph.aureus which were transferred by direct contact from the hands to the culture plates, since this represents about the maximum number of Staph.aureus aggregates which would have been transferred to another person by direct contact, as in shaking hands; on 18 of 21 occasions, from 1 to 112 Staph.aureus were transferred from the two hands, and on the other 3 occasions not any (the average number transferred per contact with the hands was 13.4).

The clothing of both carriers was found regularly to bear Staph.aureus in moderate numbers; from 1 to 134 Staph.aureus colonies were found on 25 of 29 plates scraped on the jacket, shirt front and trouser legs (the average Staph.aureus count per plate was 14.1).

The frequent presence of viable Staph.aureus on the clothing of carriers is related to the considerable resistance of this organism to drying. One experiment was made to demonstrate survival in the dried state. A sterile handkerchief was infected by a single nose-blow from Carrier D., was at once placed in a sterile jar with a loose cap, and was kept in the dark at room temperature; elution with sterile broth and making of plate counts showed that after 1 month the handkerchief still contained about 4,000,000 viable Staph.aureus/

Staph.aureus. Apart from demonstrating the prolonged survival of Staph.aureus in dried nasal secretion, this experiment showed that a nasal carrier in a single nose-blow expelled more than 4,000,000 Staph.aureus.

A handkerchief of Carrier D. after seven days of normal use, was found to contain over 10,000,000 viable Staph.aureus; another two handkerchiefs after one day of use during a cold, were found to contain, respectively, 35,000,000 and 17,000,000 Staph.aureus.

Examination of droplet spray by exposure of culture plates in front of the mouth revealed that Carrier S. did not expel Staph.aureus on any occasion in sneezing or coughing; all of 16 exposed plates were devoid of Staph.aureus. A possible reason for the absence of Staph.aureus from his droplet spray was that Carrier S. seldom carried Staph.aureus in the anterior mouth. On the other hand, Carrier D., who frequently carried a considerable number of Staph.aureus in the anterior mouth, frequently expelled Staph.aureus in coughing and sneezing; from 1 to 168 Staph.aureus colonies were found on 17 of 27 plates each exposed to a single sneeze (the average number of Staph.aureus colonies per sneeze-plate was 16.0), and from 1 to 14 Staph.aureus colonies were found on 4 of 7 plates each exposed to 12 coughs (the average number per 12-coughs-plate was 2.6).

The observations made with the slit sampler showed that sneezing seldom caused contamination of the air with/

with Staph.aureus-containing droplet nuclei, while body movement in "marching" regularly contaminated the air with Staph.aureus-carrying dust particles from the skin and clothing.

The detailed observations of droplet nucleus air infection by sneezing are shown in Table 73 for 9 experiments with Carrier D. and in Table 74 for 6 experiments with Carrier S.. In 2 of the 15 experiments a single Staph.aureus particle was found in the 10-cu.ft. "control sample" which was taken before the sneezing; this represented pre-existing dustborne contamination of the air, but of such small amount and frequency that it did not obscure interpretation of the results. The 19-cu.ft. samples taken during sneezing would contain the large settling droplets as well as the droplet nuclei of sneeze spray; these samples did not yield many more Staph.aureus than the samples taken after 1 minute after the end of sneezing, showing that sneezing did not produce a temporary air infection with large settling droplets which was greater in amount or more frequent than the droplet nucleus air infection. The 46-cu.ft. and 10-cu.ft. air samples taken after 1 minute after the last sneeze yielded few Staph.aureus and thus showed that air infection by droplet nuclei was slight and infrequent. A few Staph.aureus-containing droplet nuclei were produced on only 1 out of 9 occasions when Carrier D. gave two sneezes, and on only 2 out of 6 occasions when Carrier S. gave four sneezes./

sneezes. In the 15 experiments a total of only 16 Staph.aureus-containing droplet nuclei were found in 840 cu.ft. of air sampled out of 1500 cu.ft. exposed to 42 sneezes. It may be estimated that the 42 sneezes produced about 30 infected nuclei, less than 1 per sneeze.

The detailed observations of dustborne infection of air from skin and clothing, are shown in Table 71 for 9 experiments with Carrier D and in Table 72 for 6 experiments with Carrier S.. In only 1 of the 15 experiments did the 10-cu.ft. "control sample" taken before dust-raising show any pre-existing air infection with Staph.aureus; this only amounted to 2 infected particles per 10 cu.ft.. In all of the 15 experiments, the carrier by "marching" for 10 minutes infected the air so that some Staph.aureus were found in the 22 cu. ft. of air sampled during the 10 minutes of "marching" and the subsequent 30 minutes. In the 9 experiments with Carrier D., a total of 89 Staph.aureus-carrying dust particles were found in the 198 cu.ft. of air sampled, and in the 6 experiments with Carrier S., a total of 50 Staph.aureus-carrying particles were found in the 132 cu.ft. of air sampled. Thus, in the 15 experiments a total of 139 Staph.aureus-carrying dust particles were found in 330 cu.ft. of air sampled out of 1500 cu.ft. exposed to the carriers "marching" for 150 minutes. It may be estimated that 630 dust particles carrying Staph.aureus were liberated by the

150/

150 minutes "marching", that is over 4 per minute. Colonies of bacteria of all species were grown and counted on the heated-blood agar plates. By comparing the counts on equivalent Ludlam plates and blood plates a calculation was made of the percentage of all dustborne air bacteria comprised by Staph.aureus. In the 9 experiments with Carrier D. a total of 76 Staph.-aureus particles were found in the 90 cu.ft. of air sampled on to Ludlam plates during the 10 minutes of "marching" and the first 10 minutes of the subsequent "Die-away Period"; a total of 14,703 particles carrying bacteria of all kinds were found in 19 cu.ft. of air sampled on to heated-blood agar plates during the same period. Thus, Staph.aureus was present on about 0.11% of all bacteria-carrying dust particles liberated into the air from the skin and clothing of Carrier D.. In the 6 experiments with Carrier S. a total of 48 Staph.aureus particles were found in the 60 cu.ft. of air sampled on to Ludlam plates during the 10 minutes of "marching" and the subsequent 10 minutes; a total of 20,389 particles carrying bacteria of all kinds were found in 18 cu.ft. of air sampled on to heated-blood agar plates during the same period. Thus Staph.aureus was present on about 0.07% of all the bacteria-carrying dust particles liberated into the air from the skin and clothing of Carrier S.. Adding the results for the two carriers, it appears that Staph.aureus comprised 0.09% of all dustborne bacteria liberated into the air.

Table 71:- Staph.aureus-Carrying Dust Particles Liberated into Air of the 100 Cu.Ft. Chamber from Skin and Clothing of Nasal Carrier D. during Vigorous Bodily Activity by "Marching" for 10 Minutes.

	Air Volume Sampled	Time of Sampling in Minutes	Nine Experiments on Different Dates								
			6/5	11/5	16/5	17/5	18/5	3/6	28/6	29/6	30/6
Number of Staph. aureus on Ludlam Medium Plates per:-		Control Period:									
	10 Cu.Ft.	0 - 10	0	0	0	0	0	0	0	0	0
		Activity Period:									
	2 Cu.Ft.	11 - 13	0	0	0	2	0	1	8	3	0
	2 Cu.Ft.	14½ - 16½	1	0	1	1	1	2	11	0	1
	2 Cu.Ft.	18 - 20	0	1	3	2	0	1	12	1	0
		Die-away Period:									
	2 Cu.Ft.	22 - 24	1	0	0	2	1	0	10	1	0
	2 Cu.Ft.	26 - 28	1	1	0	0	1	0	6	0	0
	2 Cu.Ft.	31 - 33	0	0	0	0	0	0	4	0	0
	10 Cu.Ft.	41 - 51	0	0	1	1	0	0	7	0	0
	22 Cu.Ft.	Activity and Die-away Periods	3	2	5	8	3	4	58	5	1
Number of All Bacteria on Heated Blood Agar Plates per:-	½ Cu.Ft.	13½ - 14	-	709	514	609	-	602	275	650	389
	½ Cu.Ft.	17 - 17½	-	652	458	382	578	584	330	606	-
	½ Cu.Ft.	20½ - 21	-	519	483	562	488	582	477	569	289
	½ Cu.Ft.	24½ - 25	269	208	300	232	253	313	199	252	136
	½ Cu.Ft.	28½ - 29	-	249	246	84	118	-	232	221	84
	½ Cu.Ft.	33½ - 34	61	129	2	114	76	137	143	144	75
	½ Cu.Ft.	51½ - 52	16	61	4	33	51	61	49	50	50
	3½ Cu.Ft.	Activity and Die-away Periods	-	2527	2007	2016	(2000)	(2500)	1705	2492	(1300)

Table 72:- Staph.aureus-Carrying Dust Particles Liberated into Air of the 100 Cu.Ft. Chamber from Skin and Clothing of Nasal Carrier S. during Vigorous Bodily Activity by "Marching" for 10 Minutes.

	Air Volume Sampled	Time of Sampling in Minutes	Six Experiments on Different Dates: 9/6	12/6	15/6	16/6	20/6	22/6
Number of Staph. aureus on Ludlam Medium Plates per:-	10 Cu.Ft.	Control Period: 0 - 10	0	0	0	2	0	0
	2 Cu.Ft.	Activity Period: 11 - 13	0	6	1	2	2	4
	2 Cu.Ft.	14½ - 16½	2	1	1	3	1	4
	2 Cu.Ft.	18 - 20	1	6	1	1	0	4
	2 Cu.Ft.	Die-away Period: 22 - 24	0	3	0	1	0	1
	2 Cu.Ft.	26 - 28	0	3	0	0	0	0
	2 Cu.Ft.	31 - 33	0	1	0	0	0	0
	10 Cu.Ft.	41 - 51	0	1	0	0	0	0
	22 Cu.Ft.	Activity and Die-away Periods	3	21	3	7	3	13
Number of All Bacteria on Heated Blood Agar Plates per:-	½ Cu.Ft.	13½ - 14	435	228	679	870	1200	832
	½ Cu.Ft.	17 - 17½	921	264	1016	930	1754	912
	½ Cu.Ft.	20½ - 21	984	316	911	1083	1323	864
	½ Cu.Ft.	24½ - 25	523	167	487	476	744	446
	½ Cu.Ft.	28½ - 29	305	150	371	302	517	379
	½ Cu.Ft.	33½ - 34	198	112	-	238	417	116
	½ Cu.Ft.	51½ - 52	15	34	56	37	145	68
	3½ Cu.Ft.	Activity and Die-away Periods	3381	1271	(3700)	3936	6100	3617

Table 73:- Staph.aureus-Containing Droplet Nuclei Remaining in Air of the 100 Cu.Ft. Chamber after 2 'Natural Sneezes' Given in Quick Succession by Nasal Carrier D.

	Air Volume Sampled	Time of Sampling	Nine Experiments on Different Dates: 28/5	29/5	7/6	9/6	12/6	15/6	18/6	29/6	30/6	
Number of Staph. aureus on Ludlam Medium Plates per:-	10 Cu.Ft. by Slit Sampler	The 10 minutes just before sneezing.	1	0	0	0	1	0	0	0	0	Control: Dustborne Contamination
	19 Cu.Ft. by First Sieve Sampler	1 minute starting at first sneeze.	25	0	0	0	1	0	0	0	0	Infected Droplets and, possibly, Infected Droplet Nuclei.
	46 Cu.Ft. by Second Sieve Sampler	2 minutes starting at 1 minute after last sneeze.	10	0	0	0	0	0	0	0	0	Infected Droplet Nuclei.
	10 Cu.Ft. by Slit Sampler	10 minutes starting at 1 minute after last sneeze.	1	0	0	0	0	0	0	0	0	Infected Droplet Nuclei.

Table 74:- Staph.aureus-Containing Droplet Nuclei Remaining in Air of the 100 Cu.Ft. Chamber after 4 Simulated Sneezes Given in Quick Succession by Nasal Carrier S.

	Air Volume Sampled	Time of Sampling	Six Experiments on Different Dates: 16/6	18/6	22/6	23/6	24/6	25/6	
Number of Staph. aureus on Ludlam Medium Plates per:-	10 Cu.Ft. by Slit Sampler	The 10 minutes just before sneezing.	0	0	0	0	0	0	Control:- Dustborne Contamination.
	19 Cu.Ft. by First Sieve Sampler	1 minute starting at first sneeze.	1	0	0	0	0	0	Infected Droplets and, possibly, Infected Droplet Nuclei.
	46 Cu.Ft. by Second Sieve Sampler	2 minutes starting at 1 minute after last sneeze.	0	1	0	0	0	0	Infected Droplet Nuclei.
	10 Cu.Ft. by Slit Sampler	2 minutes starting at 1 minute after last sneeze.	4	0	0	0	0	0	Infected Droplet Nuclei.

Table 75:- Dissemination of Staph.aureus by Nasal Carrier D: Number of Colonies per Specimen.

Date:-	6/5	10/5	11/5	13/5	15/5	16/5	17/5	18/5	20/5	23/5	26/5
(1) Anterior Nares:-	many	many	many	many	many	many	many	many	many	many	many
(2) Throat:-	few	-	-	-	-	1	0	0	0	0	0
(3) Anterior Mouth:-	-	-	-	-	10	70	0	0	150	many	1
(4) Lips (Outside):-	-	-	-	-	-	-	-	-	-	-	-
(5) Chin:-	-	-	-	-	-	-	-	-	30	138	16
(6) Chest:-	-	-	-	-	-	-	-	-	-	0	0
(7) Leg:-	-	-	-	-	-	-	-	-	-	-	-
(8) Forearm:-	-	-	-	-	-	-	-	-	-	-	-
(9) Hands:-	2	-	-	2	1	0	59	56	3	7	0
(10) Jacket Front:-	-	-	-	-	-	1	10	5	4	1	6
(11) Jacket Sleeves:-	-	-	-	-	-	-	-	-	2	0	9
(12) Shirt Front:-	-	-	-	-	-	-	-	-	4	-	-
(13) Trouser Legs:-	-	-	-	-	-	-	-	-	1	-	2
(14) Handkerchief:-	-	-	-	-	-	-	-	-	-	-	10,000,000
(15) One Sneeze (First):-	2	0	3	0	0	0	81	168	2	0	2
(16) One Sneeze (Second):-	-	-	-	-	1	52	52	-	0	24	33
(17) 12 Coughs:-	-	-	-	-	-	-	-	-	0	1	1
(18) Droplet Nucleus Air Infection per 56 Cu.Ft. of a 100 Cu.Ft. Chamber, by 2 'NATURAL SNEEZES':-	-	-	-	-	-	-	-	-	-	-	-
(19) Dustborne Air Infection per 22 Cu.Ft. of a 100 Cu. Ft. Chamber, from Skin and Clothing by VIGOROUS BODY MOVEMENT for 10 Minutes:-	3	-	2	-	-	5	8	3	-	-	-

- means no specimen taken. (14) handkerchief after 7 days use, no cold. (Table continued overleaf):-

Table 75. continued:- Dissemination of Staph.aureus by Nasal Carrier D: Number of Colonies per Specimen.

Date:-	28/5	29/5	3/6	7/6	9/6	12/6	15/6	18/6	28/6	29/6	30/6
(1) Anterior Nares:-	many	-	many	many	many	-	-	many	-	many	-
(2) Throat:-	-	-	0	-	1	-	-	0	-	0	-
(3) Anterior Mouth:-	70	-	43	5	29	190,000/ml.	5000/ml.	0/ml.	-	40/ml.	30/ml.
(4) Lips (Outside):-	-	-	0	-	1	-	-	-	-	0	-
(5) Chin:-	-	-	4	-	-	-	-	-	-	5	-
(6) Chest:-	-	-	1	-	-	-	-	-	-	-	-
(7) Leg:-	-	-	0	-	-	-	-	-	-	0	-
(8) Forearm:-	-	-	0	-	-	-	-	-	-	0	-
(9) Hands:-	-	-	5	2	6	9	-	2	-	2	-
(10) Jacket Front:-	-	-	9	-	-	-	-	-	-	2	-
(11) Jacket Sleeves:-	-	-	2	-	-	-	-	-	-	0	-
(12) Shirt Front:-	-	-	2	-	-	-	-	-	-	0	-
(13) Trouser Legs:-	-	-	0	-	-	-	-	-	-	1	-
(14) Handkerchief:-	35,000,000	17,000,000	-	-	-	-	-	-	-	-	-
(15) One Sneeze (1st)	-	-	0	-	-	3	2	0	-	0	-
(16) One Sneeze (2nd)	-	-	1	-	-	4	1	2	-	0	-
(17) 12 Coughs:-	-	-	0	-	-	14	2	-	-	0	-
(18) Droplet Nucleus Air Infection per 56 Cu.Ft. by 2 'NATURAL SNEEZES':-	11	0	-	0	0	0	0	0	-	0	0
(19) Dustborne Air Infection per 22 Cu.Ft. by VIGOROUS BODY MOVEMENT for 10 Minutes:-	-	-	4	-	-	-	-	-	58	5	1

- means no specimen taken. (14) Handkerchief used during 1 day of an acute cold suffered from 27/5 to 2/6.

Table 76:- Dissemination of Staph.aureus by Nasal Carrier S: Number of Colonies per Specimen.

Date:-	8/6	9/6	12/6	15/6	16/6	18/6	20/6	22/6	23/6	24/6	25/6
(1) Anterior Nares:-	many	many	-	-	many	many	many	many	many	many	many
(2) Throat:-	0	0	-	-	-	20	0	20	0	3	-
(3) Anterior Mouth:-	0	0	-	-	0/ml.	10/ml.	12	0/ml.	0/ml.	0	0
(4) Lips (Outside):-	2	-	-	-	-	-	-	0	13	1	-
(5) Chin:-	130	73	-	-	-	-	-	-	6	-	-
(6) Chest:-	2	-	-	-	-	-	-	-	4	-	-
(7) Leg:-	0	-	-	-	-	-	-	-	0	-	-
(8) Forearm:-	0	-	-	-	-	-	-	-	1	-	-
(9) Right Hand:-	3	0	-	-	0	112	-	-	1	0	-
(10) Left Hand:-	-	2	-	-	1	-	-	-	6	-	-
(11) Jacket Front:-	66	-	-	-	-	-	-	-	134	-	-
(12) Jacket Sleeves:-	14	-	-	-	-	-	-	-	32	-	-
(13) Shirt Front:-	-	30	-	-	-	-	-	-	41	-	-
(14) Trouser Legs:-	22	5	-	-	-	-	-	-	4	-	-
(15) One Sneeze (First):-	0	0	-	-	-	0	0	0	0	0	-
(16) One Sneeze (Second):-	0	0	-	-	-	0	-	0	0	-	-
(17) 12 Coughs:-	0	0	-	-	-	-	0	-	0	-	-
(18) Droplet Nucleus Air Infection per 56 Cu.Ft. of a 100 Cu.Ft. Chamber, by 4 SIMULATED SNEEZES:-	-	-	-	-	4	1	-	0	0	0	0
(19) Dustborne Air Infection per 22 Cu.Ft. of a 100 Cu. Ft. Chamber from Clothing & Skin by VIGOROUS BODY MOVEMENT for 10 Minutes:-	-	3	21	3	7	-	3	13	-	-	-

- means no specimen taken.

(15), (16) & (17) were counts on plates exposed in front of mouth.

Section 18: Discussion and conclusions.

These observations on two "nasal carriers" of Staph.aureus yield information about the relative probability of infection being spread by each of the different possible routes. They show that it is possible for Staph.aureus to be transmitted by contact, by the projection droplets of cough spray and sneeze spray, by airborne droplet nuclei from sneezing, and by airborne dust particles from the skin and clothing.

The demonstration of air infection with droplet nuclei containing Staph.aureus is of great interest because, in spite of droplet nucleus air infection having been hypothesised for over 15 years (Wells, 1934), there has not been reported previously any clear demonstration of the production of droplet nuclei which contained pathogenic organisms.

None of the different mechanisms of infection were found to bring about transmission of more than a small number of Staph.aureus. Only small numbers of Staph.aureus were obtained by contact from the hands, lips, chin, chest, leg, forearm, jacket, shirt and trousers of the carriers; the observations disprove the common belief that "contact infection" involves the transmission of especially large numbers of the specific pathogenic organism.

Immediate spray infection, by the projection droplets of coughing and sneezing, appeared rather less likely than "contact infection"; in coughing and

sneezing/

sneezing, one carrier expelled only a few Staph.aureus-containing droplets and the other carrier did not expel any.

The amount of air infection produced by the carriers was not very great, never more than one or two Staph.aureus particles per cu.ft. of air in the 100 cu.ft. chamber. However, these few airborne infected particles would have an excellent chance of being inhaled by persons near the carrier, and thus of reaching the nasal passages, their most favourable habitat. In contrast, the few Staph.aureus which were transmitted by "contact" or by "projection droplets" would reach, in the first place, only the skin and clothing of the recipient, and could be transmitted to the nose or mouth only by further transfer by the hands, by eating utensils or by secondary air infection; only a small proportion of the few Staph.aureus received by by the recipient would be likely ultimately to reach his respiratory tract. If, as is generally believed, Staph.aureus infection is initially nasal, the present findings show that airborne inhalation infection is the most likely mode of spread. If, on the other hand, infection can primarily become established on the skin and the Staph.aureus multiply freely on the skin, it may be concluded from the present findings that infection is as likely to occur by "contact" and by "projection droplets" as by air carriage of the staphylococcus.

The/

The observations make it clear that liberation of dust from skin and clothing is a much more important cause of air infection than spray emission by sneezing. On average, less than 1 Staph.aureus-containing droplet nucleus was put into the air per sneeze, while more than 4 Staph.aureus-carrying dust particles were put into the air per minute of "marching" by the carrier. Unfortunately, measurements were not made of air infection produced by shaking of the carrier's handkerchief. Possibly this would have been much greater in amount than the air infection produced by the carrier "marching". Dumbell, Lovelock and Lowbury (1948) found that shaking of a dry used handkerchief liberated into the air, on average, about 15,000 bacteria-carrying particles; they reported that many Staph.aureus were included among the bacteria set free, but had not made counts of these.

Among the potential sources of infected dust, very large numbers of Staph.aureus were found only on the carrier's handkerchief; relatively small numbers of Staph.aureus were found on different parts of the skin and clothing. Different methods were employed for observation of the Staph.aureus, in the case of the handkerchief, the skin and the clothing, so that direct comparison of the counts is not warranted. It does appear, however, that the handkerchief is the most prolific source of infected dust. On the other hand, shaking of the handkerchief is a relative infrequent event as compared with movement of skin and clothing.

Section 19: Summary of Part 4.

(a) Observations were made on the cough spray of 20 adult male patients with open pulmonary tuberculosis. The cough droplets were collected on a (3 in. x 1 in.) microscope slide held at 3 inches in front of the mouth, and were examined microscopically after staining by the Ziehl-Neelsen method; this procedure was estimated to collect about a quarter of the infected droplets which were expelled. Droplets containing M. tuberculosis were found to be expelled by 10 out of the 20 patients in giving 6 coughs. Of 410 droplets collected from the 120 coughs, 36 were found to contain tubercle bacilli: 19 droplets each containing between 1 and 10 tubercle bacilli, 9 droplets each containing between 10 and 100 tubercle bacilli, 3 droplets each containing between 100 and 1000 tubercle bacilli, and 5 droplets each containing between 5000 and 40,000 tubercle bacilli. The deposit marks of a quarter of the droplets which contained tubercle bacilli, were less than 120 microns in diameter and these would have remained airborne as infected droplet nuclei; thus, a small amount of air infection would have been produced by the coughing of some of the patients.

Swabs taken before coughing from the throat and anterior mouth were treated with acid and cultured on egg medium. It was found that, before coughing, tubercle bacilli were present in the throat secretions of 15 of the 20 patients, and in the anterior mouth secretions/

secretions of 10 of the 20 patients; presumably the throat and mouth had been contaminated with infected sputum coughed up from the lungs on a previous occasion. Of the 10 patients who expelled infected droplets, all carried tubercle bacilli in the throat and 8 carried tubercle bacilli in the anterior mouth; it was concluded that the infected droplets originated from the throat and mouth, and not directly from the bronchi.

(b) Observations were made on the cough spray of 50 adult and child patients with faucial diphtheria. The cough droplets were collected on a 10-sq.in. plate of Hoyle's medium held at 3 inches in front of the mouth, this being judged capable of catching most of the infected droplets expelled. Droplets containing C.diphtheriae were found to be expelled by 10 out of the 50 patients in giving 6 coughs. The 300 coughs expelled a total of 48 C.diphtheriae-containing droplets (0.16 per cough) and 1106 other droplets containing bacteria cultivable on Hoyle's medium (i.e. less than 4.2% of the droplets contained C.diphtheriae).

Swabs taken from the anterior mouth before the coughing were cultured on Hoyle plates. It was found that 12 of the 50 patients carried a few C.diphtheriae in the anterior mouth secretions. In coughing, C.diphtheriae-containing droplets were expelled by 6 of the 12 patients with anterior mouth infection (in all, 43 infected droplets), but by only 4 of the 38 patients without/

without anterior mouth infection (in all, only 5 infected droplets); probably most of the infected cough droplets originated from the anterior mouth.

(c) Observations were made on the cough spray of adults and children infected with Strept.pyogenes, including 50 scarlet fever patients and 37 chronic tonsillitis patients and "healthy throat carriers". The cough droplets were collected on a 10-sq.in. blood agar plate held at 3 inches in front of the mouth. Droplets containing Strept.pyogenes were found to be expelled during 6 coughs by 39 out of the 87 persons with infected throats; the 522 coughs expelled a total of 1109 Strept.pyogenes-containing droplets (2 per cough) and 8220 droplets carrying bacteria of other kinds (i.e. 12% of the collected droplets contained Strept.pyogenes). Swabs taken from the anterior mouth before coughing were cultured on blood agar plates. It was found that only 13 of the 87 persons with throat infection carried Strept.pyogenes in the anterior mouth secretions. In coughing, Strept.pyogenes-containing droplets were expelled by 5 of the 13 persons with anterior mouth infection (in all, 63 infected droplets) and by 34 of the 74 persons without anterior mouth infection (in all, 1046 infected droplets); either, many of the infected droplets originated directly from the throat, or originated from the anterior mouth after its contamination with secretion brought forward from the throat by the first coughs of the series.

Three/

Three special experiments were made to test the relative importance of the different air infecting mechanisms: droplet spray, liberation of dust from clothing and raising of dust from floor and furniture.

i) The droplet spray from 4 "healthy throat carriers" of Strept.pyogenes who coughed, talked, sang and laughed during 100 minutes while sitting at a game of cards in the centre of a large room (16,000 cu.ft.), was found to cause very little infection of the air in the near vicinity (on average, 0.8 per cu.ft. of Strept.pyogenes-carrying particles)

ii) In contrast, very heavy infection of the air was caused by liberation of dust from the skin, clothing, towels and handkerchiefs of "healthy throat carriers" of Strept.pyogenes. In a small (1960 cu.ft.) disinfected room, four carriers, masked to prevent droplet spray, by marching to and fro during 6 minutes increased the Strept.pyogenes content of the air by 1.4 per cu.ft. (i.e. by at least 120 infected particles per carrier per minute of marching); then, by shaking vigorously their jackets, towels and handkerchiefs during 16 minutes, they increased the Strept.pyogenes content of the air by about 200 per cu.ft. (i.e. by at least 6000 infected particles per carrier per minute of shaking). After shaking, heavy air infection persisted for some time; 100 Strept.pyogenes particles per cu.ft. was found 20 minutes later. This long duration of air carriage proved that many of the infected/

infected particles were small in size (probably from 1 to 10 microns in diameter).

iii) Moderately heavy infection of the air was caused by raising of dust from the floor and bedding of a large dormitory which was normally occupied by 18 persons, including 6 "healthy throat carriers" of Strept.pyogenes. Six "non-carriers" by marching up and down the dormitory during 13 minutes raised very little infected dust from the floor (on average, 0.1 Strept.pyogenes particles per cu.ft.); by sweeping the floor with sterile brushes during 17½ minutes they increased the Strept.pyogenes content of the air to an average of 4.9 per cu.ft., and by shaking blankets, pillows and towels during 18 minutes they increased it again from zero to an average of 4.7 per cu.ft.

(d) A variety of bacteriological observations were made on 2 "healthy nasal carriers" of Staph.aureus on, respectively, 22 and 11 days during 8 weeks in the summer; cultures were made on the selective medium of Ludlam (1949) and only coagulase-positive Staph.aureus colonies were counted. On all occasions, both carriers bore large numbers of Staph.aureus in the anterior nares; it was thought that the nose was the true habitat of the Staph.aureus and that the presence of Staph.aureus on other parts of the body represented only a temporary contamination from the nose. Rarely the carriers bore a few Staph.aureus in the throat. The anterior mouth secretion of one carrier (S.) sometimes/

sometimes contained a few Staph.aureus (e.g. 10 per ml.) and that of the other (D.) usually contained a few Staph.aureus (30, 40, 5000 and 190,000 per ml. on four occasions).

Not much infection was found to be disseminated by the projection droplets of coughing and sneezing. In the case of one carrier (S.), Staph.aureus was not found on any of 16 plates each exposed at 3 inches in front of the mouth to one sneeze or 12 coughs. In the case of the other carrier (D.), Staph.aureus was found on the majority of the plates exposed to a sneeze (on 17 of 27 plates) or to 12 coughs (on 4 of 7 plates); on average, 16 Staph.aureus-containing droplets were collected per sneeze and 0.2 per cough.

Observations were made with a slit sampler in a 100-cu.ft. test chamber occupied by the carrier; these showed that sneezing seldom contaminated the air with Staph.aureus-containing droplet nuclei, while the body movements of "marching" regularly contaminated the air with Staph.aureus-carrying dust particles from the skin and clothing.

Air contamination with Staph.aureus-containing droplet nuclei was demonstrated under experimental conditions designed to preclude air contamination with dust from skin, clothing, floor or walls. One carrier (D.) by 2 sneezes infected the air in only 1 of 9 tests; the other carrier (S.) by 4 sneezes infected the air in 2 of 6 tests. In the 15 tests a total of only

16/

16 Staph.aureus-containing droplet nuclei were found in 840 cu.ft. of air sampled out of 1500 cu.ft. exposed to 42 sneezes; it was estimated that the 42 sneezes produced about 30 infected nuclei, less than 1 per sneeze.

Air contamination with Staph.aureus-carrying dust particles from the skin and clothing was demonstrated under experimental conditions designed to preclude air contamination by droplet spray or by dust raising from floor and walls. By 10 minutes of "marching", the air was infected in all of 9 tests with one carrier (D.) and in all of 6 tests with the other carrier (S.). In the 15 tests, a total of 139 Staph.aureus-carrying dust particles were found in 330 cu.ft. of air sampled out of 1500 cu.ft. exposed to "marching" by a carrier for 150 minutes; it was estimated that about 630 infected dust particles were liberated into the air by the 150 minutes of "marching", that is more than 4 per minute. Staph.aureus was found on 0.09% of all the bacteria-carrying dust particles liberated from the skin and clothing of the two carriers. In 4 of the 15 tests, Staph.aureus-carrying dust particles were found in the air at later than 20 minutes after cessation of the "marching"; this long duration of air carriage proved the small size of some of the infected particles (probably from 1 to 10 microns in diameter).

In the case of both carriers a few Staph.aureus usually were found on the jacket front, jacket sleeves, shirt/

shirt front, trouser leg, hands and chin; Staph.aureus seldom was found on the lips, chest, forearm and leg. The number of Staph.aureus recovered from these surfaces by swabbing and by direct application of culture plates was taken as representing the number which might be transferred to another person by direct "contact", and this number was found to be small. Contact with the two hands transferred some Staph.aureus to a culture plate on 18 out of 21 occasions; the number of Staph.aureus transferred per contact varied from 0 to 112, being 13.4 on average. Similar numbers of Staph.aureus were transferred from the clothing of the carriers to a culture plate on 25 out of 29 occasions; the average number of Staph.aureus collected per plate was 14.1. It was concluded that "direct contact infection" normally involves the transfer of only a very few Staph.aureus cell aggregates.

Elution and making of plate counts showed that three handkerchiefs of one of the carriers (D.), after a few days use (during a cold in two cases), contained respectively 10,000,000, 17,000,000 and 35,000,000 living Staph.aureus. A sterile handkerchief was infected by a single nose-blow from this carrier; after standing dry in a sterile jar at room temperature in the dark during 1 month, this handkerchief was found to contain 4,000,000 living Staph.aureus. Heavy air infection is likely to result from the shaking of handkerchiefs containing so many Staph.aureus.

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Part 5

INVESTIGATION OF THE NATURAL BACTERIAL CONTENT OF AIR IN OCCUPIED PREMISES WITH ESPECIAL REFERENCE TO STREPT. PYOGENES AND STAPH. AUREUS.

CONTENTS OF PART 5

Section 16:/

Section 1: General introduction.

Demonstration of the natural occurrence of pathogenic bacteria in air is the best possible proof that infection may be transmitted by the aerial route. Enumeration of the pathogenic bacteria in air enables comparison with the numbers found on other possible vehicles of infection and so yields information as to the relative probability of infection occurring by air carriage. From knowledge of the average concentration of pathogenic bacteria in the air of a room or building it is possible to calculate the inhalation dose, the number of pathogenic bacteria which will be inhaled by a person remaining in this place for a given time. For proper calculation of the average level of air contamination which will be experienced by the occupants of a certain place, it is necessary to measure the general level of air contamination in the place on many separate occasions. Accordingly, in the present investigation, many air samples were taken in each room, at different times and on different days. The air sampler was placed near the centre of the room, the occupants were not allowed to congregate near it and no one except the observer was allowed to approach to nearer than about 3 feet from it. This ensured that each air sample was fairly representative of the room as a whole and was unlikely to be loaded by some high localised and temporary concentration of bacteria liberated from an infected person in the immediate vicinity.

By/

By correlating the observed extent of bacterial contamination of the air with the circumstances at the time of taking each sample, it is possible to learn how air infection is influenced by such factors as ventilation, number of occupants, walking, talking, coughing, bed-making and sweeping. The important causes of air infection may be indicated in this way. For this reason, the circumstances at the time of taking each sample were recorded.

Pathogenic bacteria are rarely present in air in more than very low concentrations and they comprise only a small proportion of the total aerial flora. Failure has often attended attempts to demonstrate the presence of pathogenic bacteria in the air of infected premises, because the air has been sampled in insufficient amount or by an inefficient method; when large volumes of air are sampled on to generally favourable culture media the pathogenic bacteria may be overgrown by the more numerous saprophytic organisms. To ensure success in the present investigation the very efficient slit sampler of Bourdillon, Lidwell and Thomas (1941) was used, large volumes of air were sampled by taking many samples on to separate culture plates, and selective culture media were used to reduce the growth of saprophytic organisms.

The investigation of Strept.pyogenes air infection in a training institution was made in collaboration with Dr.C.A.Green and Dr.S.W.Challinor, and that of Staph. aureus air infection in a hospital with Dr.A.T.Wallace.

Part 5a

Bacterial Content of Air in a Residential Training Institution during an Epidemic of Throat Infections with Strept.pyogenes.

Section 2: Account of the institution and the epidemic.

The institution provided training in mechanical engineering for 765 men, aged 15 to 19 years. The community was housed and trained in a permanent camp of single-storied buildings, complete with dormitories, mess rooms, kitchens, workshops, schoolrooms, swimming baths, recreation rooms, cinema, and gymnasium. Each dormitory, room and hall was a separate building, and these buildings were linked together by covered corridors. The nearest village was one mile away, and at four miles there was a fairly large town. The men were allowed to visit both places in their leisure time, but relatively few took the opportunity. The trainee population joined the institution in groups of 50 to 70 at intervals of four to six months and remained for three and a half years.

The health of the community was very good during the summer months of 1942, but about the middle of October, tonsillitis became prevalent and was accompanied by the appearance of scarlet fever. A rise in the incidence of common colds had preceded the tonsillitis wave by some weeks. By November 7th there had been 49 cases of tonsillitis and 16 cases of scarlet fever. Between November 8th and 21st there were 26 further cases of tonsillitis and 13 of scarlet fever. The investigation was made between November 13th and 29th, that is at the height of the epidemic. At the time of the investigation the incidence of healthy "throat carriers"/

carriers" of Strept.pyogenes in one of the badly affected sections of the community was about 30%; probably the carrier rate in the other sections was similarly high.

Neither before nor at the time of the epidemic in the institution was there any evidence of an epidemic in the adjacent village and town.

Section 3: Experimental methods.

The bacterial content of air was examined by use of a slit sampler constructed to the design of Bourdillon, Lidwell and Thomas (1941). The slit sampler was operated to take 1 cubic foot of air per minute. The slit-plate distance was 3 millimeters instead of the usual 2 millimeters. The air intake of the sampler was situated at 3½ feet above the floor.

Some of the air samples were taken on to plates of ordinary blood agar (nutrient agar with 5% horse blood) and others on to plates of blood agar incorporating crystal violet in a concentration of 1 in 1000,000. Both these media allowed good growth of the streptococci and development of their characteristic haemolysis. Colonies of Strept.pyogenes were readily recognised by the beta haemolytic zones; it was thus easy to count the Strept.pyogenes colonies separately from the colonies of other bacteria. A wide variety of air bacteria were able to grow aerobically on the ordinary blood agar plates; the total colony counts obtained on these plates were taken as representing the numbers of "all bacteria" present in the air. The crystal violet blood agar was inhibitory to the majority of the air bacteria other than the streptococci; this medium facilitated examination of the air for its streptococcus content by allowing the sampling of larger volumes of air on to each plate without danger of overcrowding by saprophytic bacteria. Usually, 3-cu.ft. amounts of air/

air were sampled on to the ordinary blood agar plates and 6-cu.ft. or 12-cu.ft. amounts on to the crystal violet blood agar plates.

The plates were incubated aerobically at 37 deg.C. for about 24 hours. After scoring of the agar surface by parallel cuts with a scalpel blade, the colonies on each plate were counted with the aid of a binocular (x 20) plate microscope. Use of the microscope was necessary for sure recognition of the smaller colonies, some of which were barely visible to the naked-eye (colonies of diphtheroid bacilli) and were difficult to distinguish from dust particles unless magnified. Incubation for periods longer than 24 hours was found to give some increase in colony size, but it resulted in frequent spoiling of plates by "spreaders"; for the latter reason, counts were made after incubation for only 24 hours.

Counts were made on each culture plate, of (1) all colonies present, (2) beta haemolytic colonies which resembled colonies of Strept.pyogenes, and (3) alpha haemolytic colonies which resembled colonies of Strept. viridans. The results were expressed as the number of bacteria-carrying particles per cubic foot of air; this corresponds to the number which would be inhaled by a resting adult in about 3 minutes. In the tables of results the time is shown at which the taking of each sample was begun.

A proportion of the beta haemolytic colonies were filmed,/

filmed, tested for soluble haemolysin production, grouped by Lancefield's method and typed by slide agglutination. Almost all the beta haemolytic colonies examined were found to be soluble haemolysin producing streptococci of Group A; they are referred to as Strept.pyogenes. Typing by slide agglutination showed that the majority of these streptococci belonged to Griffith's Type 1.

The observations were made as follows. The observer entered the room at a time when it was not occupied. He installed the slit sampler in a central position. He made observations at intervals during (1) an initial period before occupation, when air infection was minimal, (2) the period of occupation by the usual occupants of the room, when there were varying degrees of crowding and movement and, thus, varying degrees of air infection, and (3) a period following occupation, when air infection diminished. In addition to taking air samples, the observer noted the number of occupants in the room, the ventilation and the amount and kind of movement and activity. The observer only approached closely to the slit sampler when changing the culture plates or adjusting the air flow; in doing this he was careful to avoid talking and unnecessary movement, so as to minimise air infection from his own person. At all other times the observer sat quietly at about 6 feet from the sampler. The occupants of the room were forbidden to congregate near to the sampler in order to watch its operation.

Section 4: Observations in a dormitory.

The dormitory examined was one used by members of the section of the community which suffered most heavily during the epidemic. Of the 24 men using the dormitory, 6 were absent in hospital having contracted tonsillitis or scarlet fever; of the remaining 18, a third were "throat carriers" of Strept.pyogenes.

The dormitory was 16,000 cu.ft. in capacity (80 ft. x 20 ft. x 10 ft.). Apart from the single-beds and clothing lockers, no other furniture was present. The wooden floor was covered with polished linoleum; this was swept daily. Good ventilation was secured by means of windows down each side of the room. Most of the windows were partly open throughout the 24 hours during which observations were made. Even at night when the black-out curtains were drawn, a slight draught was noticeable near the windows. The slit sampler was placed in the centre of the dormitory at about 8 feet from the foot of the nearest beds.

Observations were made throughout a continuous period of 24 hours during which the usual occupants of the dormitory were present at the customary times and behaved in their customary manner. In all, 75 air samples were taken successfully, comprising a total of 451 cubic feet of air. These observations are recorded in Table 77. They fell into 6 periods.

(1) In the Afternoon Period, from 3.30 to 5.00 p.m., the dormitory was unoccupied except for three observers who/

who did not make much movement. The amount of air infection was small; on average, there were 0.05 per cu.ft. (1 in 19 cu.ft.) of Strept.pyogenes, and 7 per cu.ft. (50 in 7 cu.ft.) of "all bacteria".

(2) In the Evening Period, from 5.00 to 9.30 p.m., there was occupation of the dormitory by a number of men varying between 1 and 18, with frequent entering and leaving. For the most part, the men sat reading or talking. From time to time they undertook more vigorous activities such as changing clothes or dancing. There was some coughing. Between 9.00 and 9.30 p.m. the men undressed and retired to bed. The amount of air infection was considerably greater than in the Afternoon Period; on average, there were 0.3 per cu.ft. (25 per 87 cu.ft.) of Strept.pyogenes, and 72 per cu.ft. (1086 per 15 cu.ft.) of "all bacteria". There was an increase of air infection between 9.00 and 9.30 p.m. when the men were undressing and going to bed.

(3) In the Night Period, from 9.30 p.m. to 6.00 a.m., the 18 men were asleep in bed and the observer was seated quietly at 6 feet from the slit sampler. The amount of bacterial contamination of the air was very small; on average, there were 0.01 per cu.ft. (2 in 186 cu.ft.) of Strept.pyogenes, and 11 per cu.ft. (201 in 18 cu.ft.) of "all bacteria". It is of great interest that air infection was so very low during this period when the maximum number of occupants were present, but movement, talking and other activities were/

were minimal. Following the cessation of movement and activity at between 9.30 and 9.40 p.m., there was a rapid reduction in the previously high level of air infection, a reduction of about 85% in half an hour of all airborne bacteria cultivable on ordinary blood agar or on crystal violet blood agar.

(4) In the Early Morning Period, from 6.00 to 8.30 a.m., the number of occupants and the amount and kind of activity varied greatly. The activities included dressing, entering and leaving the dormitory, brushing of shoes, bed-making, sweeping of the floor, singing and coughing. The amount of bacterial contamination of the air was very great; on average, there were 0.19 per cu.ft. (11 in 60 cu.ft.) of Strept.pyogenes, and 145 per cu.ft. (873 in 6 cu.ft.) of "all bacteria". When reveille was sounded at 6.00 a.m., all the men at once jumped from bed and started to dress hurriedly. This sudden burst of activity caused an immediate increase in air infection which was observed in the sample taken between 6.00 and 6.06 a.m.. The large bacterial content of the air occurring in the period of dressing and bed-making (6.00 to 6.30 a.m.) was rapidly reduced after 6.30 a.m. when most of the men departed to breakfast; within half an hour there was a reduction of 94% in all airborne bacteria cultivable on crystal violet blood agar. At 7.30 a.m. and later, the air infection was again increased; this increase corresponded with bed-making and sweeping of the floor

by/

by the few remaining occupants. An especially high level of air infection was observed at 8.00 a.m.; this may have been due to sweeping in the vicinity of the slit sampler or to the movements of all the occupants at that time returning together into the dormitory. This high concentration of bacteria in the air was reduced rapidly in the subsequent quieter period; within half an hour there was a 98% reduction in the number of airborne bacteria cultivable on crystal violet blood agar.

(5) In the Late Morning Period, from 8.30 to 10.30 a.m., there were few occupants and little movement. Air infection was slight; on average, there were 0 per cu.ft. (0 in 42 cu.ft.) of Strept.pyogenes, and 5 per cu.ft. (30 in 6 cu.ft.) of "all bacteria".

(6) In the Mid-day Period, from 10.30 a.m. to 2.00 p.m., there was a varying number of occupants and varying activity which included standing and talking, sitting and playing cards, changing and brushing clothes, and coughing. Air infection was moderately great; on average, there were 0.09 per cu.ft. (5 in 57 cu.ft.) of Strept.pyogenes, and 33 per cu.ft. (498 in 15 cu.ft.) of "all bacteria".

In all periods taken together a total of 44 Strept. pyogenes particles were found in 451 cubic feet of the air of the dormitory. Of "all bacteria" cultivable on ordinary blood agar plates, 2738 were found in 67 cu.ft. of air. Of the Strept.pyogenes colonies, 12 were found/

found on the ordinary blood agar plates used for sampling these 67 cu.ft. of air. Strept.pyogenes thus comprised only about 0.5% of all the airborne bacteria. With regard to all bacteria cultivable on crystal violet blood agar, 543 were found in 384 cu.ft. of air. Alpha haemolytic colonies resembling colonies of Strept.viridans comprised 75% of these colonies growing on the crystal violet blood agar plates.

Section 5: Observations in the cinema hall.

The cinema hall was a large and lofty room, being 67,000 cu.ft. in capacity (90 ft. x 50 ft. x 15 ft.). Ventilation did not appear adequate. All windows were closed and draped with black-out curtains. Two doors were opened only intermittently. The air became oppressive and the temperature rose 6 deg.F. during one hour when the room was occupied. About 300 men, from all sections of the community, constituted the audience on the occasion of the investigation. These men sat on wooden benches arranged at either side of a central passage which was 12 feet wide. The slit sampler was installed in the middle of this passage.

Observations were made throughout a period of 5½ hours, before, during and after the performance. In all, 33 air samples were taken, comprising a total of 183 cubic feet of air. These observations are recorded in Table 78; they fall into three sections.

(1) In the period Before Occupation, from 6.30 to 8.00 p.m., only between 2 and 4 persons were present in the hall; these did not make much movement. Air infection was slight; on average, there were 0.02 per cu.ft. (1 in 51 cu.ft.) of Strept.pyogenes and 10 per cu.ft. (126 in 12 cu.ft.) of "all bacteria".

(2) In the period During Occupation, from 8.00 to 11.00 p.m., the film was exhibited and many men were present in the hall. The men entered during the half hour between 8.00 and 8.30 p.m.. About 300 men were present/

present during the performance from 8.30 to 10.40 p.m.. There was some coughing and laughing during the performance, and some movement in and out of the hall during each of the three intervals. Most of the audience departed from the hall between 10.40 and 10.50 p.m.. The floor was swept and benches were moved between 10.50 and 11.00 p.m.. Air infection was great during the period of occupation; on average, there were 0.33 per cu.ft. (31 in 93 cu.ft.) of Strept. pyogenes, and 62 per cu.ft. (931 in 15 cu.ft.) of "all bacteria". The highest levels of air infection corresponded in time with the entry of the audience, and with exit of the audience and sweeping of the hall.

(3) In the period After Occupation, from 11.00 to 12.00 p.m., the hall was unoccupied except for the observer. Air infection was again very low; on average, 0 per cu.ft. (0 in 39 cu.ft.) of Strept.pyogenes, and 26 per cu.ft. (79 in 3 cu.ft.) of "all bacteria". The high bacterial content of the air occurring at the end of the period of occupation was rapidly reduced after departure of the occupants; within half an hour there was a reduction of 92% in the number of airborne bacteria cultivable on crystal violet blood agar.

In all periods taken together, 32 Strept.pyogenes particles were found in 183 cubic feet of air. With regard to "all bacteria" cultivable on ordinary blood agar, 1136 were found in 30 cu.ft. of air. There were

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5 Strept.pyogenes colonies on the ordinary blood agar plates used to sample these 30 cu.ft. of air; Strept. pyogenes thus comprised only about 0.5% of all the air bacteria. With regard to all bacteria cultivable on crystal violet blood agar, 458 were found in 153 cu.ft. of air. Alpha haemolytic colonies resembling colonies of Strept.viridans comprised 74% of all the colonies on the crystal violet blood agar plates.

Section 6: Observations in a schoolroom.

The schoolroom was large, 30,000 cu.ft. in volume (100 ft. x 25 ft. x 12 ft.). All the windows were closed, and the two doors were opened only occasionally. Ventilation seemed adequate and the air did not become oppressive. The desks and benches were wooden, and the floor was of polished wood. The pupils were 52 men; they were seated on benches which were distributed throughout the room. The class was divided into three sections, each in charge of a separate master. The masters walked about and talked a great deal. There was little talking and coughing by the pupils. The slit sampler was installed a little to one side of the centre of the room. Observations were made throughout a period of 3 hours, before, during and after the holding of a class. In all, 19 air samples were taken, comprising a total of 121 cubic feet of air. These observations are recorded in Table 79; they fall into three sections.

(1) In the period Before Occupation, from 5.30 to 5.45 p.m., the room was unoccupied except for the two observers who made little movement. Air infection was slight; on average, there were 0 per cu.ft. (0 in 12 cu.ft.) of Strept.pyogenes.

(2) In the period During Occupation, from 5.45 to 7.45 p.m., the class was held and the schoolroom was occupied by 57 pupils, masters and observers. The amount of bacterial contamination of the air was large; on/

on average, there were 0.63 per cu.ft. (53 in 85 cu.ft.) of Strept.pyogenes, and 26 per cu.ft. (309 in 12 cu.ft.) of "all bacteria". The especially high level of air infection found in the last sample, taken between 7.37 and 7.43 p.m., was correlated with the disturbance among the pupils preparing to leave.

(3) In the period After Occupation, from 7.45 to 8.30 p.m., the room was unoccupied except for one observer. A considerable air contamination with Strept.pyogenes persisted for an unusually long period after the termination of occupation; on average during the 45 minutes after occupation, there were 0.54 per cu.ft. (13 in 24 cu.ft.) of Strept.pyogenes. On the other hand, there was a rapid reduction in the total bacterial content of the air; within half an hour after the end of the class, there was a 90% reduction in the airborne bacteria cultivable on crystal violet blood agar.

In all periods taken together, 66 Strept.pyogenes particles were found in 121 cubic feet of air. With regard to "all bacteria" cultivable on ordinary blood agar, 309 were found in 12 cu.ft. of air. There were 4 Strept.pyogenes colonies on the ordinary blood agar plates used to sample these 12 cu.ft. of air. Thus, Strept.pyogenes comprised 1.3% of all the airborne bacteria. With regard to all bacteria cultivable on crystal violet blood agar, 180 were found in 109 cu.ft. of air. Alpha haemolytic colonies resembling colonies of Strept.viridans comprised 50% of these latter.

Section 7: Observations in a recreation room.

The recreation room had a capacity of 32,400 cu.ft. (90 ft. x 30 ft. x 12 ft.). The floor was covered with linoleum. The windows were kept shut, the door was opened only occasionally and four fan-ventilators were in very slow operation. Ventilation appeared inadequate, since the air quickly became oppressive on occupation of the room. The slit sampler was placed somewhat to one side of the centre of the room. Observations were made throughout a period of 4 hours, before, during and after occupation of the room by a large number of men. In all, 23 air samples were taken, comprising a total of 120 cu.ft. of air. These observations are recorded in Table 80; they fall into three sections.

(1) In the period Before Occupation, from 4.30 to 5.00 p.m., there were only between 1 and 4 persons in the room and these made little movement. Air infection was slight; on average, there were 0 per cu.ft. (0 in 21 cu.ft.) of Strept.pyogenes, and 7 per cu.ft. (59 in 9 cu.ft. of "all bacteria".

(2) In the period During Occupation, from 5.00 to 8.20 p.m., many men were present in the room and a variety of activities were undertaken. The men were drawn from all sections of the community. The number present varied mainly between 50 and 150; the average number present was probably about 75. Men were frequently entering and leaving the room. Most men spent/

spent their time in the recreation room mainly in sitting on the benches which were scattered about all parts of the room, smoking, talking or playing cards. Some men danced or played table tennis. The amount of bacterial contamination of the air was large; on average, there were 0.38 per cu.ft. (34 in 93 cu.ft.) of Strept.pyogenes, and 65 per cu.ft. (973 in 15 cu.ft.) of "all bacteria".

(3) In the period After Occupation, from 8.20 to 8.35 p.m., very few occupants remained in the room. Air was infected with Strept.pyogenes to the extent of 0.3 per cu.ft. (2 in 6 cu.ft.).

In all the periods taken together, 36 Strept.pyogenes particles were found in 120 cubic feet of air. With regard to "all bacteria" cultivable on ordinary blood agar, 1032 were found in 24 cu.ft. of air. There were 6 Strept.pyogenes colonies on the ordinary blood agar plates used to sample these 24 cu.ft. of air. Thus Strept.pyogenes comprised only 0.6% of all the airborne bacteria. With regard to all bacteria cultivable on crystal violet blood agar, 181 were found in 96 cu.ft. of air. Alpha haemolytic colonies resembling colonies of Strept.viridans comprised 79% of all colonies on the crystal violet blood agar plates.

Table 77:- Number of Bacteria-Carrying Particles per Cubic Foot of Air of the 16,000 Cu.Ft. Dormitory at Various Times during 24 Hours of Normal Occupation by 18 Men of whom 6 were Strept.pyogenes 'Carriers'.

Circumstances	Time	Cu.Ft. Sampled	Numb. of Occupants	Number per Cu.Ft. of		
				All Bact. on BA	All on CVBA	Strept. pyogenes
Afternoon Period few occupants, little movement	3.30	6	3	-	0.7	0.2
	3.40	4	3	8	-	0.0
	3.45	6	3	-	0.0	0.0
	3.55	3	3	6	-	0.0
Evening Period:- varying degree of occupation and movement; men sit, read, talk, cough, change clothes, dance etc. from time to time during evening.	5.20	6	11	-	0.3	0.0
	5.30	3	14	27	-	0.0
	5.40	6	9	-	1.3	0.7
	6.00	6	16	-	3.3	0.3
	6.20	6	15	-	1.3	0.2
	6.30	3	12	48	-	0.0
	6.40	6	12	-	1.7	0.2
	7.00	6	11	-	1.5	0.0
	7.20	6	13	-	2.0	0.0
	7.30	3	13	51	-	1.7
	7.40	6	13	-	1.3	0.0
9.00-9.30: men undress and retire to bed.	8.00	6	13-1	-	1.5	0.3
	9.00	6	14	-	5.0	0.2
	9.06	3	16	93	-	0.0
	9.10	6	18	-	4.7	0.5
At 9.30 p.m. all in bed.	9.20	6	18	-	5.1	1.0
	9.30	3	18	143	-	0.0
Night Period:- all occupants in bed and quiet from 9.30 to 6.00. Observer sits quietly at 6 feet from slit sampler.	9.40	6	19	-	1.2	0.0
	10.00	6	19	-	0.8	0.2
	10.10	3	19	22	-	0.0
	10.30	6	19	-	0.2	0.0
	10.40	3	19	11	-	0.0
	11.00	6	19	-	0.2	0.0
	11.30	6	19	-	0.2	0.0
	11.40	3	19	6	-	0.0
	12.00	6	19	-	0.7	0.0
	12.30	12	19	-	0.5	0.0
	1.00	12	19	-	0.3	0.0
	1.30	12	19	-	0.0	0.0
	1.45	3	19	5	-	0.0
	2.00	12	19	-	0.1	0.0
	2.30	12	19	-	0.1	0.0
	3.00	12	19	-	0.3	0.0
	3.30	12	19	-	0.3	0.0
	3.45	3	19	7	-	0.0
	4.00	12	19	-	0.3	0.0
	4.30	12	19	-	0.2	0.0
	5.00	12	19	-	0.6	0.0
	5.30	12	19	-	0.2	0.0
	5.45	3	19	16	-	0.2

(Table continued overleaf)

Table 77, continued:-

Circumstances	Time	Cu.Ft Sampled	Numb. of Occupants	Number per Cu.Ft. of All Bact. on BA	All on CVBA	Strept. pyogenes
Early Morning:-	6.00	6	19	-	4.3	0.2
All rise at 6.00	6.15	6	10	-	-	-
and dress, make	6.30	6	8-4	-	7.7	0.5
beds, go out &	6.40	3	3	187	-	0.7
return;	6.45	6	2	-	1.8	0.0
much coughing &	7.00	6	7-1	-	0.5	0.0
movement,	7.15	6	3	-	0.7	0.0
6.45-7.15, quiet.	7.30	6	4	-	3.2	0.0
Bedmaking from	7.40	3	4	104	-	0.3
7.15 to 7.45.	7.45	6	4	-	2.2	0.0
Floor swept at	8.00	6	2-19	-	18.3	0.7
7.45 to 8.00.	8.15	6	19	-	2.0	0.0
Late Morning:-	8.30	6	1	-	0.3	0.0
Few occupants.	8.40	3	3	5	-	0.0
Observer mainly	8.45	6	1	-	0.5	0.0
alone.	9.00	6	1	-	0.0	0.0
Door and windows	9.30	6	1	-	1.7	0.0
open.	9.40	3	2	5	-	0.0
	10.00	6	4	-	0.5	0.0
	10.30	6	4	-	1.3	0.0
Mid-day Period:-	10.40	3	7	44	-	0.7
Changing and	11.00	6	5	-	0.3	0.0
brushing clothes	11.30	6	5	-	0.8	0.0
at 10.40-11.00.	11.40	3	5	5	-	0.3
Playing cards	12.00	6	1	-	0.2	0.0
at 12.00-12.30.	12.30	6	1-10	-	1.2	0.0
Walking and	12.36	3	15	21	-	0.0
talking from	12.40	6	20	-	0.8	0.2
12.30 to 2.00.	12.46	3	20	19	-	0.0
	12.50	6	20	-	1.2	0.0
	1.45	3	20	77	-	0.3
	2.00	6	20	-	2.2	0.0

BA:- blood agar
CVBA:- blood agar with 1 in 1000,000 crystal violet.

Note:- of all the colonies on the crystal violet blood agar plates, 75% were alpha haemolytic and resembled the colonies of Strept.viridans.

Table 78:- Number of Bacteria-Carrying Particles per Cubic Foot of Air of the 67,500 Cu.Ft. Cinema Hall at Various Times Before, During and After Occupation by an Audience of 300 Men Including Many 'Carriers' of Strept.pyogenes.

Circumstances	Time	Cu.Ft. Sampled	Numb. of Occupants	Number per Cu.Ft. of		
				All Bact. on BA	All on CVBA	Strept. pyogenes
Before Occupation	6.30	6	3	-	0.8	0.2
	6.38	6	4	16	-	0.0
few occupants;	6.47	6	4	-	0.3	0.0
little movement	6.55	9	2	-	0.0	0.0
	7.06	6	2	-	0.0	0.0
	7.14	6	4	6	-	0.0
	7.30	6	4	-	0.2	0.0
	7.38	6	4	-	0.5	0.0
During Occupation	8.00	6	6	-	0.8	0.0
	8.08	3	12	17	-	0.3
audience enter	8.15	6	100	-	4.2	0.5
at 8.00-8.30;	8.30	6	300	-	8.2	0.7
	8.45	6	300	-	5.0	0.7
sit watching	9.00	6	300	-	3.3	0.7
cinema show	9.08	3	300	63	-	1.0
at 8.30-10.40,	9.22	6	300	-	1.8	0.2
with some	9.30	6	300	-	2.0	0.5
coughing and	9.38	3	300	67	-	0.0
laughing;	9.45	6	300	-	8.0	0.2
	10.08	3	300	50	-	0.3
audience leave	10.15	6	300	-	4.3	0.2
at 10.40-10.50	10.23	6	300	-	3.2	0.2
	10.30	6	300	-	7.3	0.3
	10.38	3	300	113	-	0.0
floor swept at	10.45	6	→12	-	5.7	0.3
10.53.	10.53	6	3	-	12.0	0.0
After Occupation	11.00	6	1	-	5.3	0.0
	11.15	6	1	-	1.3	0.0
observer alone.	11.23	6	1	-	1.0	0.0
	11.30	6	1	-	0.8	0.0
	11.38	3	1	26	-	0.0
	11.45	6	1	-	0.2	0.0
	12.00	6	1	-	0.0	0.0

BA:- Blood agar.
CVBA:- Blood agar with 1 in 1000,000 crystal violet.

Note:- Of all the colonies on the crystal violet blood agar plates, 74% were alpha haemolytic and resembled colonies of Strept.viridans.

Table 79:- Number of Bacteria-Carrying Particles per Cubic Foot of Air of the 30,000 Cu.Ft. Schoolroom at Various Times Before, During and After Occupation by a Class of 55 Men Including Some 'Carriers' of Strept.pyogenes.

Circumstances	Time.	Cu.Ft. Sampled	Numb. of Occupants	Number per Cu.Ft. of		
				All Bact. on BA	All on CVBA	Strept. pyogenes
Before Occupation observers alone.	5.30	6	2	-	0.0	0.0
	5.37	6	2	-	0.2	0.0
During Occupation	5.45	6	57	-	1.5	0.2
	5.53	7	57	-	1.2	0.4
class seated	6.06	6	57	26	-	0.3
and quiet;	6.12	12	57	-	1.2	0.3
only the three	6.26	6	57	-	1.0	0.5
masters walk	6.33	6	57	-	0.8	0.3
about and talk.	6.40	6	57	-	1.3	0.2
	6.47	6	57	26	-	0.3
	6.54	6	58	-	2.2	1.5
class leaves at	7.15	6	57	-	0.8	0.3
7.42-7.44.	7.22	6	57	-	1.8	1.5
	7.30	6	57	-	1.8	1.2
	7.37	6	57	-	7.7	1.2
After Occupation	7.45	6	1	-	4.2	1.0
	8.00	6	1	-	1.3	0.3
observer alone.	8.15	6	1	-	0.7	0.2
	8.30	6	1	-	1.2	0.7

BA:- Blood agar.
CVBA:- Blood agar with 1 in 1000,000 crystal violet.

Note:- Of all the colonies on the crystal violet blood agar plates, 50% were alpha haemolytic and resembled colonies of Strept.viridans.

Table 80:- Number of Bacteria-Carrying Particles per Cubic Foot of Air of the 32,400 Cu.Ft. Recreation Room at Various Times Before, During and After its Occupation by 50 to 150 Men Including Many 'Carriers' of Strept.pyogenes.

Circumstances	Time.	Cu.Ft. Sampled	Numb. of Occupants	Number per Cu.Ft. of		
				All Bact. on BA.	All on CVBA.	Strept. pyogenes
Before Occupation	4.30	6	1	-	0.2	0.0
few occupants,	4.36	6	4	6	-	0.0
little movement.	4.49	6	4	-	0.3	0.0
	4.57	3	2	7	-	0.0
During Occupation	5.15	6	80	-	2.8	0.3
	5.22	3	100	111	-	0.3
most men sit and	5.30	6	100	-	1.3	0.3
read, smoke or	5.38	3	80	78	-	1.0
talk; some sing	5.45	6	60	-	1.3	0.0
dance or play	5.55	3	50	35	-	0.3
table tennis;	6.00	6	50	-	0.8	0.5
	6.07	3	50	31	-	0.0
	6.21	6	80	-	1.4	0.2
atmosphere soon	6.28	3	80	70	-	0.3
becomes very	6.43	6	70	-	0.8	0.0
oppressive &	7.00	6	70	-	2.8	0.8
smoke laden;	7.15	6	100	-	1.5	0.2
	7.30	6	130	-	1.5	0.3
occupants leave	7.45	6	150	-	2.0	0.2
between 7.45	8.00	6	few	-	4.2	0.5
and 8.20.	8.10	6	few	-	4.3	0.5
	8.20	6	few	-	3.7	0.8
After Occupation	8.30	6	few	-	1.2	0.3

BA:- Blood agar.
CVBA:- Blood agar with 1 in 1000,000 crystal violet.

Note:- Of all the colonies on the crystal violet blood agar plates, 79% were alpha haemolytic and resembled colonies of Strept.viridans.

Table 81:- Average Numbers of Bacteria-Carrying Particles per Cubic Foot of Air in the Dormitory, Cinema Hall, Schoolroom and Recreation Room before and during Occupation by Men of whom Some Were 'Carriers' of Strept.pyogenes.

Organism	Period	Dormitory	Cinema Hall	Schoolroom	Recreation Room
Strept.pyogenes	Before Occupation	0.05	0.02	0.00	0.00
	During Occupation	0.22*	0.33	0.63	0.38
All Bacteria	Before Occupation	7	10	-	7
	During Occupation	74*	62	26	65
Average number of occupants during period of occupation:-		12	300	57	75
Average number of occupants per 1000 cubic feet:-		0.8	3.8	1.9	2.3
Movement and Activity:-		Much	Moderate	Little	Moderate
Ventilation:-		Great	Poor	Moderate	Poor

*wakeful occupation only

Section 8: Amount of air contamination with Strept. pyogenes and size of inhalation dose.

The chief interest of these observations relates to the numbers of Strept.pyogenes which were found in the air. A total of 875 cubic feet of air from the dormitory, cinema hall, schoolroom and recreation room was found to contain 178 particles carrying Strept.pyogenes, that is 0.2 per cu.ft. of air. In different 3-cu.ft. and 6-cu.ft. samples the content of Strept.pyogenes particles varied from 0 to 1.7 per cu. ft.. The Strept.pyogenes particles comprised 0.5% of all bacteria-carrying particles in the air; out of 5215 colonies of all kinds found on ordinary blood agar plates, 27 were colonies of Strept.pyogenes.

Of the 875 cubic feet of air sampled, 475 cubic feet were taken from rooms during normal occupation by many members of the community, and 400 cubic feet were taken from rooms when few or no occupants were present (214 cu.ft.) or when, in the dormitory at night, all occupants were asleep in bed (186 cu.ft.). This sampling ratio of 4¾ parts of heavily contaminated air to 4 parts of slightly contaminated air was thought to correspond approximately with the division of the trainee's daily timetable between time spent exposed to highly infected air (i.e. when in rooms occupied at the same time by many others) and time spent not exposed to highly infected air (i.e. when in rooms with few occupants, when sleeping in the dormitory, when walking out/

out of doors and when taking leave from the institution). For these reasons, it is considered that the 875 cubic feet of air sampled was a typical "cross-section" of the atmospheric environment encountered daily during the height of the epidemic by inmates of the institution. Thus, the observed average air content of 0.2 Strept.pyogenes particles per cu.ft. was accepted as a basis for calculating the numbers of Strept.pyogenes which were inhaled by persons in the institution. Assuming that an adult inhales 500 cubic feet of air per day in normal breathing, a person living in the institution at the height of the epidemic must have inhaled about 100 particles carrying Strept.pyogenes per day. A stranger entering the premises of the institution would have inhaled a Strept.pyogenes particle within about 15 minutes of entry.

The present observations thus indicate the frequency of inhalation infection and the approximate size of the inhaled dose. The exact number of the inhaled Strept.pyogenes cells can not be calculated, because the number of Strept.pyogenes carried on each airborne particle is not known. It seems probable that most of the infected particles found in the air did not carry more than a few Strept.pyogenes cells. The Strept.pyogenes cells in discharged respiratory tract exudate can rarely exceed 100,000,000 per cubic centimeter; Hamburger (1944) found that the saliva of/

of persons with throat infection usually contained between 1000 and 1000,000 Strept.pyogenes per c.c. (as colony forming units, i.e. probably single cells and chains of up to 10 or 20 cells). The solid content of saliva and similar secretions is about 1%. Thus, the maximum Strept.pyogenes content of 100,000,000 cells per c.c. of liquid secretion is equivalent to 100,000,000 cells per 0.01 c.c of dried secretion residue, that is 1 cell per 100 cubic microns of residue. For infected particles consisting of secretion residue attached to cloth fibres, skin scales and other dust fragments, the maximum Strept.pyogenes content will be even less than 1 per 100 cubic microns. The mean diameter of the bacteria-carrying particles found in the air of the institution was probably in the region of 10 microns; this size would accord with the frequently observed air infection die-aways of 90% in half an hour, which suggest a mean sedimentation rate of about 0.002 meters per second (calculated by formula of Phelps and Buchbinder, 1941). Very few of the bacteria-carrying particles found in the air could have been larger than 100 microns in diameter, since particles of this size fall to the floor within a few seconds after their liberation into the air of a room. Accepting the calculated maximum rate of 1 cell of Strept.pyogenes per 100 cubic microns of dried secretion residue, it is concluded that an airborne particle of 10 microns diameter (1000 cubic microns if cubical/

cubical) would contain not more than 10 Strept.pyogenes cells, and a particle of 100 microns diameter (1000,000 cubic microns if cubical) not more than 10,000 Strept.pyogenes cells. Thus it is thought that of the Strept.pyogenes-carrying particles found in the air of the institution, most carried only between 1 and 100 Strept.pyogenes cells, and none carried more than 10,000. An inmate of the institution inhaling 100 infected particles per day would be acquiring between 100 and 10,000 Strept.pyogenes cells per day.

This estimated daily inhalation dose refers to the numbers of Strept.pyogenes acquired in breathing the air at large in rooms of the institution; it does not include the numbers of Strept.pyogenes which might be inhaled by a person encountering a specially high concentration of air infection in the immediate vicinity of a "carrier" or other source of infection.

It must be emphasised that the air infection measurements which have been presented, averaging 0.2 Strept.pyogenes particles per cu.ft., relate to an advanced stage of the epidemic, to the sixth and seventh weeks of the epidemic. By this time many prolific sources of air infection had developed: many "carriers" and various heavy accumulations of Strept.pyogenes in floor dust, bedding and personal clothes. Earlier in the epidemic, the sources of air infection must have been fewer and less productive, and thus the average level of Strept.pyogenes air contamination must have been less.

Section 9: Role of airborne infection in spread of epidemic.

Having obtained these measurements of air contamination with Strept.pyogenes, it is of interest to consider the part played by airborne infection in the spread of the epidemic. Other possible mechanisms of transmission are by food or milk, by immediate droplet spray, and by contact, directly or indirectly by fomites. In the present case, the possibility of food or milk infection had been considered by the medical officer of the institution and excluded on epidemiological grounds. Among the causes of droplet spray, talking was frequent, coughing fairly frequent, but sneezing apparently very rare inspite of the contemporary incidence of common colds (only one sneeze was heard during observation in the four rooms). There were frequent opportunities for transmission as the result of indirect contact between these young men living, working, eating and playing together in common rooms: for instance in the common handling of doors and chairs, in the common use of bathroom facilities, in the sharing of tools and in the exchange of books and magazines.

In assessing the relative importance of each of the possible mechanisms of transmission, particular consideration must be given to the frequency of transmission and the number of Strept.pyogenes transmitted.

The/

The frequency of occurrence of a given mechanism of transmission must be considered in relation to the incidence of infection, Thus, the rapid spread of the epidemic, the large numbers of cases of tonsillitis and scarlet fever, and the high "carrier" rate, are evidence that a ready and frequent mechanism of transmission was in operation. It is not possible that the epidemic spread was brought about mainly by an infrequent mechanism of transmission such as some mode of immediate contact involving an obvious and gross breach of hygiene and decent behaviour (e.g. by the licking of a spoon just after its use by another). On the other hand, inhalation of airborne Strept.pyogenes, droplet spray production by talking and coughing, and indirect contact by handling of common objects, were each of such frequent occurrence that, as regards frequency, any one of these modes of transmission could by itself have accounted satisfactorily for the epidemic incidence of infections.

In the case of airborne infection, estimates have been obtained of the average daily dose. As shown in Section 8, every person living in the institution at the height of the epidemic must have inhaled per day about 100 infected particles carrying a total of between 100 and about 10,000 Strept.pyogenes cells. The epidemiological significance of these figures can not be deduced with certainty, because the smallest number of Strept.pyogenes which will initiate infection of/

of the human respiratory tract, the minimum infecting dose, is not known. On the one hand, it may be argued that an inhalation dose of 100 to 10,000 Strept.pyogenes cells is not sufficient to initiate either clinically apparent infection or commensal colonisation of the respiratory tract, that airborne infection therefore did not play an important part in the spread of the epidemic, and that infection was transmitted by other mechanisms supposed capable of delivering much larger numbers of Strept.pyogenes to the respiratory tract. On the other hand, it may be argued that an inhalation dose of 100 to 10,000 Strept.pyogenes cells is sufficient to initiate infection or commensal colonisation, and therefore, in view of the numbers of Strept.pyogenes found in the air of the institution, that airborne infection was largely responsible for the spread of the epidemic. For various reasons, the latter view is preferred and is accepted provisionally as a basis for discussion. It is thought that a single particle carrying a few Strept.pyogenes cells may be sufficient in many persons on many occasions to initiate infection or commensal colonisation of the respiratory tract, and consequently that the present epidemic was spread mainly by airborne infection.

In the first place it may be stated that there is no existent evidence which suggests that there is a definite minimum size of infecting dose such that 10,000, 100 or even 1 Strept.pyogenes cell would usually/

usually fail to initiate infection, while a larger number, say 1000,000 or more, would usually succeed. Almost certainly, resistance to infection varies very greatly between different persons, and also, in one person, between different areas of the respiratory tract mucous membrane and between different times, according to circumstances such as fatigue, body temperature and air temperature. It seems unlikely that, in face of such variations in resistance, the minimum infecting dose could remain sufficiently constant in size for infection to be caused frequently by 1000,000 or more Strept.pyogenes and very rarely by 100 or 10,000 Strept.pyogenes.

The second reason for believing that infection may result frequently from inhalation of a few Strept.pyogenes cells, is that a much larger number of cells could not have been acquired regularly by any of the other possible mechanisms of transmission which occurred frequently enough to account for the high incidence of infections in the epidemic. Thus, from a consideration of the observations recorded in Parts 2 and 4, it is thought that the number of Strept.pyogenes which could be transmitted in the droplet nuclei of immediate, close-range cough spray and speech spray, would be very small. Immediate droplet spray infection with large projection droplets might transmit larger numbers of Strept.pyogenes, but only on to the skin and clothing of the recipient; no more than a small proportion/

proportion of these would ultimately find their way into the mouth or nose. The frequently occurring indirect contacts between different persons, as by the handling of objects used in common, were not likely to result in more than a few Strept.pyogenes cells reaching the respiratory tract of the recipient. Hamburger and Green (1946) found the average number of Strept.pyogenes on the hands of "nose carriers" to be 790,000 (0 to 21,000,000) and of "throat carriers" to be 4700 (0 to 250,000). Obviously, only a small proportion of these streptococci will be transferred by simple contact from the skin of the carrier's hand on to any object, only a small proportion of those on the infected object will be transferred to the hands of a second person, and only a small proportion of those reaching the hands of this recipient will be passed to the respiratory mucosa on handling of his mouth or nose. Even if Strept.pyogenes were present on many objects handled by the recipient and even if the recipient was in the habit of putting his hand to his mouth at frequent intervals, the autodisinfecting power and retentiveness of the skin make it unlikely that the hand would act as a vehicle for the introduction of very large numbers of Strept.pyogenes into the respiratory tract. It is of course possible, by exceptionally direct and intimate contact, for very large numbers of Strept.pyogenes to be acquired. Thus, if the infector, a "carrier", contaminated a spoon or

other/

other object with highly infected secretion, containing perhaps 100,000,000 Strept.pyogenes per c.c., and if a second person, the recipient, immediately afterwards put this spoon or object into his mouth, he might acquire a large number of Strept.pyogenes, perhaps 10,000,000 in 0.1 c.c. of secretion. However, such gross breaches of decent behaviour can not have been sufficiently common to have accounted for the high incidence of infection in the present epidemic.

If, as these arguments indicate, infection often resulted from inhalation of a single infected particle carrying a few Strept.pyogenes cells, airborne infection must have been a major cause, if not the main cause of spread of the epidemic. All 765 members of the community must have inhaled such a dose of Strept.pyogenes many times a day during several weeks; probably all received Strept.pyogenes for the first time long before the beginning of the investigation on November 13th. Whether inhalation of an infected particle resulted in scarlet fever, tonsillitis, "throat carriage", "nose carriage" or complete elimination of the Strept.pyogenes within a few hours, would depend on the contemporary state of the recipient's general immunity and of the local defence mechanisms of the area of mucous membrane inoculated. Some persons contracted a clinically apparent infection for the first time at a late stage in the epidemic; there were 26 fresh cases of tonsillitis and 13 of scarlet/

scarlet fever following the start of the investigation on November 13th, by which time the epidemic was a month old. Presumably these persons had acquired Strept.pyogenes, but resisted infection, on many earlier occasions, and had succumbed to infection with Strept.pyogenes acquired on a later occasion when for some reason the respiratory mucosa had a lessened power of resistance. It is not thought that these persons had acquired Strept.pyogenes for the first time at a late stage in the epidemic.

As discussed in Section 8, the average amount of air infection must have been much less in the early stages of the epidemic when the sources of air infection were fewer. Whether, in the early stages, infection was transmitted mainly through the air or mainly by some other means can only be guessed. Although the total Strept.pyogenes output of one or a few carriers might not be sufficient to raise significantly the general level of air infection in a large room, this output may produce a high level of air infection in the carriers' immediate vicinity for a short period of time; for instance, four carriers in a small room (1960 cu.ft.), by shaking their jackets, towels and handkerchiefs, produced a temporary air contamination amounting to 50 Strept.pyogenes particles per cu.ft. per carrier (see Part 4). In this way, a single carrier may by the aerial route infect one or a few persons who happen to be standing close by him while he undertakes an air infecting activity.

Section 10: Cause of Bacterial Contamination of the Air.

It was hoped that the sources and causes of bacterial contamination of the air might be discovered by correlating the contamination level with the contemporary circumstances of crowding, ventilation, personal movement, talking, coughing, bed-making, sweeping and other activities. Only partial success attended this aspect of the investigation. The main relationships are shown in Table 81, which summarises the average findings for the periods "before occupation" and "during occupation" for each of the four rooms examined.

The observations made one thing clear beyond all doubt: namely, that air infection depended upon occupation. Bacterial contamination of the air was very slight when the room was unoccupied and had been unoccupied for some time, as in the period "before occupation" (the presence of one to three uninfected observers was neglected). Contamination was great, 5 to 10 times greater, when the room was occupied by many members of the community, as in the period "during occupation". After termination of occupation, this great contamination of the air rapidly became less and reached a low level within about half an hour (e.g. 10% of the level "during occupation"). This correlation of air infection with occupation was found in the case of all of the four rooms and in the case both of Strept.pyogenes and of "all bacteria".

The sole exception to correlation between occupation of the room and high air contamination, was very instructive. At night in the dormitory when all occupants were present, but asleep in bed, bacterial contamination of the air was very slight, as slight as when the dormitory was unoccupied. This observation proves that it is not the mere presence of occupants which produces bacterial contamination of air, but rather the wakeful activities of the occupants.

Comparison between the different rooms did not yield much information beyond an additional indication that air infection depends more on the kind and amount of activity of the occupants than on the number of occupants. There was surprisingly little difference between the four rooms in the amount of bacterial contamination of the air. In the dormitory, cinema hall, schoolroom and recreation room, the average bacterial contents of the air were, respectively, 0.22, 0.33, 0.65 and 0.38 per cu.ft. of Strept.pyogenes and 74, 62, 26 and 65 per cu.ft. of "all bacteria". The differences in amount of air infection, such as they were, could not be correlated with the differences in number of occupants, in degree of crowding or in amount of ventilation. The cinema hall and the recreation room were the most badly crowded and the most poorly ventilated, yet these rooms had less air contamination with Strept.pyogenes than the less crowded and better ventilated schoolroom, and less air contamination/

contamination with "all bacteria" than the much less crowded and much better ventilated dormitory.

Unfortunately, ventilation measurements were not made. Ventilation was judged roughly according to the number of windows and doors observed open, any draught felt, smell and the extent of temperature rise during occupation. The difference between the well ventilated dormitory and the poorly ventilated cinema hall and recreation room, was obvious and marked. The dormitory was at all times cool and fresh, its windows were always open and draughts were often felt. The cinema hall and recreation room became hot and stuffy during occupation; their windows were kept closed. Inspite of this, bacterial contamination of the air was as great in the dormitory as in the cinema hall and recreation room. It is concluded that ventilation is a factor of only minor importance in determining the level of air infection.

Little information was obtained by correlating the air infection levels in individual air samples with the contemporary circumstances. Separate peak contaminations were not found relating to different activities such as coughing, talking, walking, dressing, sweeping and bed-making. These activities were not sufficiently isolated; different activities occurred at the same time and overlapped in such a way that their detailed correlation with the level of air infection was not possible. As a rule, however, air infection peaks

were/

were observed when the total amount of activities of all kinds was at a maximum; for instance, in the dormitory when the men were retiring to bed (9.00 to 9.35 p.m.), when they were rising and dressing (6.00 to 6.45 a.m.) and when they were making beds, sweeping the floor, moving about and coughing a great deal (7.40 to 8.06 a.m.); and in the other rooms at the beginning and end of the period of occupation when many men were walking about (entering or leaving the room), benches were being moved and the floor was being swept.

Section 11: Value of crystal violet blood agar as a selective culture medium for Strept.pyogenes from air.

Blood agar containing gentian violet in a concentration of 1 in 500,000 was recommended by Garrod (1933) as a useful selective medium for isolation of haemolytic streptococci. This medium was employed by Thomas for sampling the air of occupied rooms ; it was found to give good growth of Strept.pyogenes and to inhibit colony formation by 95% of the airborne saprophytic bacteria (Bourdillon, Lidwell and Thomas, 1941). Challinor (1943) recommended use of a related dye, crystal violet, in a concentration of 1 in 1000,000. In the present investigation, a trial was made of this latter medium. Plates of blood agar with 1 in 1000,000 of crystal violet and plates of ordinary blood agar were both used for air sampling. The samples taken on each medium were distributed in a comparable manner throughout the different experiments and the different periods of each experiment. Thus, the characteristics of the media may be compared in terms of the total colony counts obtained on each.

Ordinary blood agar was used in 39 plates to sample a total of 133 cu.ft. of air; these plates bore 5215 colonies of all kinds, which included 27 colonies of Strept.pyogenes.

Crystal violet blood agar was used in 111 plates to sample a total of 742 cu.ft. of air; these plates bore 1362 colonies of all kinds, which included 151 colonies of Strept.pyogenes.

Thus,/

Thus, ordinary blood agar bore Strept.pyogenes colonies at the rate of 0.20 per cu.ft. of air (27 from 133 cu.ft.), while crystal violet blood agar bore Strept.pyogenes colonies also at the rate of 0.20 per cu.ft. of air (151 in 742 cu.ft.). This surprisingly exact equality in the Strept.pyogenes recovery rate between ordinary blood agar and crystal violet blood agar, suggests that the crystal violet does not inhibit growth of any significant number of Strept.pyogenes. Blood agar incorporating 1 in 1000,000 of crystal violet seems fully as favorable for recovery and growth of airborne Strept.pyogenes as ordinary blood agar.

Colonies of bacteria of all kinds ("all bacteria") were found on ordinary blood agar at the rate of 39.2 per cu.ft., and on crystal violet blood agar at the rate of 1.84 per cu.ft.. Thus, of "all bacteria" growing on ordinary blood agar, only 4.7% grew on crystal violet blood agar. Crystal violet inhibited growth of 95% of the air bacteria other than Strept. pyogenes.

Strept.pyogenes colonies comprised 0.5% of all colonies on ordinary blood agar, but 11.1% of all colonies on crystal violet blood agar.

Alpha haemolytic colonies resembling colonies of Strept.viridans comprised 3.3% of all colonies on ordinary blood agar, but 72% of all colonies on crystal violet blood agar. It is probable that a large proportion of these colonies were not Strept.viridans, but/

but "alpha micrococci".

This investigation shows that the following advantages attach to the use of crystal violet blood agar in examining air for Strept.pyogenes: while crystal violet in a concentration of 1 in 1000,000 does not inhibit any Strept.pyogenes, it inhibits and suppresses growth of 95% of the airborne bacteria of other species, so that it is possible without causing overcrowding and confluence of colonies to take on to crystal violet blood agar plates air samples which are 20 times larger than can be taken on to ordinary blood agar plates.

Part 5b

Bacterial Content of Air in a Maternity Hospital Having Frequent Staph.aureus Infections.

Section 12: Account of maternity unit and infection incidence.

The investigation was conducted between March 12th and July 2nd, 1947, in the maternity unit of a large general hospital. The maternity unit consisted of offices, a wash room, two nursery wards for the babies, and several wards for the mothers. These different rooms were on the same floor, opening on to a common corridor. This corridor was also connected to the rest of the hospital.

Bacteriological examination of the air was carried out in four of the rooms, namely Nursery A, Nursery B, a Mothers' Ward and the Wash Room.

Nursery A was a large airy room of 5500 cu.ft. capacity (30 ft. x 14 ft. x 13 ft.). It had four south-facing windows and one door. The floor was polished wood. There were 26 cots in Nursery A; the majority of these, always over half of them, were occupied by babies.

Nursery B was a little smaller, being 3700 cu.ft. in capacity (18 ft. x 16 ft. x 13 ft.). It had one east-facing double window and one door. Its floor was polished wood. The cots numbered 14, and the majority of these were occupied throughout the investigation.

The Mothers' Ward was a large airy room of 5400 cu.ft. capacity (26 ft. x 16 ft. x 13 ft.). It had two south-facing and three east-facing windows, and two doors. The floor was polished wood. There were

9 beds, most or all of which were occupied.

The Wash Room was about 1000 cu.ft. in capacity. It was entered frequently by nurses for washing of linen and rinsing of utensils.

The babies remained for the most part of the day in their cots in the Nurseries. The mothers remained in the Mothers' Ward. The babies were brought into the Mothers' Ward at four-hourly intervals for feeding. The Nurseries and the Mothers' Ward were entered frequently by doctors, nurses and domestic servants. The common activities were medical examinations, bed-making, "bed-panning", serving of meals, toilet, sweeping and dusting.

The Nurseries and the Mothers' Ward were well ventilated. The windows and doors were usually partly open during the daytime. The air was judged "fresh" at all times.

Mothers and their babies remained in the hospital only for a few weeks, so that there was a rapid turnover of the population.

In the months prior to March 12th, 1947, the maternity unit had reported a high incidence of Staph.aureus infections among both mothers and babies. Staph.aureus infections continued to occur throughout the 3½ month period of the investigation. Among the babies, in this period, there were several cases of Staph.aureus conjunctivitis, several cases of "septic spots" on the skin infected with Staph.aureus, and one case/

case of fatal Staph.aureus pneumonia. Among the mothers, there was one case of Staph.aureus breast abscess, one case of a boil due to Staph.aureus, one case of Caesarian operation wound infection with Staph.aureus and one case of Staph.aureus conjunctivitis; in addition, Staph.aureus was found in small numbers in high vaginal swabs taken from 13 mothers.

In addition to this high incidence of clinically apparent Staph.aureus infection, there was a high rate of "healthy carriage" of Staph.aureus among the babies, mothers and nurses. For instance, on one occasion (July 1st), swabs were taken from both nostrils of 7 nurses and 26 babies. The presence of Staph.aureus was demonstrated in 2 of the nurses (29%) and in 11 of the babies (42%).

Section 13: Experimental methods.

Two methods were used for examining the bacterial content of the air, namely exposure of culture plates to direct sedimentation of infected particles ("settling plates"), and use of the Bourdillon slit sampler.

The "settling plates" had a diameter of 3½ inches and presented about 10 square inches of culture medium surface. They were left open lying on a table at about 3 feet above the floor and in a central position in the room.

The slit sampler, constructed according to the design of Bourdillon, Lidwell and Thomas (1941), was run with a slit-plate distance of 2 millimeters and at an air-flow of 1 cubic foot per minute. The slit sampler was placed centrally in the room; its air-intake was about 3 feet above the floor. Persons other than the observer were forbidden to approach the slit sampler closely.

Four different culture media were used: (1) plain nutrient agar (1% meat extract, 1% peptone, ½% sodium chloride and 2% agar), (2) ordinary blood agar (nutrient agar and 5% horse blood), (3) MacConkey's bile-salt neutral-red lactose peptone agar, and (4) salt-milk medium (nutrient agar with 20% milk and 7% sodium chloride). The nutrient agar and the blood agar were regarded as generally favorable culture media. The MacConkey's medium and the salt-milk medium were thought/

thought to be somewhat selective for Staph.aureus; they are inhibitory to bacteria of many other species and they enhance development of the golden pigment which is characteristic of Staph.aureus. It was intended, by using these media in parallel to compare their value in the examination of air for Staph.aureus.

Plates were incubated aerobically at 37 deg.C. for 24 hours and were then left at room temperature for a further 24 hours. Each plate was scored by parallel cuts with a scalpel blade and observed with a (x 20) binocular plate microscope; the total number of colonies of all kinds was counted. The plate was then examined with the naked-eye for golden colonies resembling Staph.aureus; all such colonies were subcultured and tested for coagulase production. Only coagulase-positive strains were counted as Staph.aureus.

Section 14: Observations made with 24-hour settling plates in two nurseries, a mothers' ward and a wash room.

A series of observations was made with settling plates. A MacConkey plate and a salt-milk medium plate were placed side by side in each of the rooms being examined. They were left uncovered continuously throughout a 24-hour period. This procedure was repeated on each of 40 days chosen at intervals during the period 12/3/47 to 2/7/47. On each day a pair of plates were exposed in Nursery A, in Nursery B and in either the Mothers' Ward (14 days) or the Wash Room (23 days). The numbers of coagulase-positive Staph.aureus found on these 24-hour, 10-sq.in. plates are shown in Table 82.

The number of particles carrying Staph.aureus which settled in 24 hours on a 10 sq.in. plate varied on different days between 0 and 7. Staph.aureus was found in at least one room on 36 out of the 40 days. It was found with more or less similar frequency throughout all periods of the investigation, except for the three weeks from 30/5/47 to 23/6/47 when it was found with a somewhat greater frequency than at the other times.

Staph.aureus was found with equal frequency in the four different rooms; for the whole investigation, the average number of Staph.aureus found per 24-hour 10-sq. in. plate was 0.97 (73 per 75 plate-days) in Nursery A, 0.87 (66 per 76 plate-days) in Nursery B, 0.88 (23 per 26 plate-days)/

26 plate-days) in the Mothers' Ward, and 0.80 (33 per 41 plate-days) in the Wash Room.

Staph.aureus was found with almost equal frequency on MacConkey Plates as on salt-milk plates. For the whole investigation, the average number of Staph.aureus found per 24-hour MacConkey plate was 0.85 (99 per 117 plate-days) and per 24-hour salt-milk plate was 0.95 (96 per 101 plate-days). The proportion of all colonies on each plate comprised by Staph.aureus was the same for the MacConkey medium as for the salt-milk medium (see Table 82, bottom row). These findings indicate that MacConkey medium and salt-milk medium are of equal value in examining air for Staph.aureus.

Presumably the total number of colonies developing on these selective media was somewhat less than the total number of bacteria-carrying particles settling on the plates. Accordingly, the percentage of the colonies which were found to be Staph.aureus (see Table 82, bottom row), must be greater than the percentage of all airborne bacteria comprised by Staph.aureus. It is concluded that Staph.aureus particles comprised less than 1% of all bacteria-carrying particles settling from the air.

A total of 195 Staph.aureus colonies were found on 218 plates each exposed for 24 hours in one of the four rooms. Thus, the average deposition rate of Staph.aureus particles in the maternity unit was 0.9 per 10-sq. in. plate per 24 hours, or 0.037 per plate per hour.

Section 15: Observations made with slit sampler and settling plates in the two nurseries and mothers' ward.

In the second series of observations the slit sampler and settling plates were used in parallel. In each experiment, observations were made continuously for 5½ to 6½ hours in the morning between 7.30 a.m. and 2.00 p.m.. Observations were made on two days in Nursery A, on two days in Nursery B and on three days in the Mothers' Ward. In 6 of these 7 experiments, MacConkey plates were exposed in the slit sampler; in 1 experiment (in the Mothers' Ward), nutrient agar plates were exposed in the slit sampler. On each plate in the slit sampler, a 25-cu.ft. air sample was taken during the first 25 minutes of each successive half hour, the last 5 minutes of the half hour being allowed for the changing of plates; about 300 cu.ft. of air was taken by the slit sampler each morning. Settling plates were exposed beside the slit sampler; two 10-sq.in. MacConkey plates were exposed continuously throughout the whole 5½ to 6½ hours of the experiment, and one blood agar plate was exposed during the first 25 minutes of each half hour (a fresh blood agar plate each half hour). Except when changing the culture plates and adjusting the sampler, the observer stood outside the room being examined so that he would not himself contribute to the air infection. He was dressed in his ordinary clothes covered by a sterile surgical gown, and wore a sterile cap and mask. A

record/

record was made of the number of times that a nurse or any other person entered the room; the number of such "entrances" during the time of sampling was taken as a rough measure of the amount of activity in the room during that time.

The detailed findings for each of the 7 experiments are shown in Tables 83-89, and the average findings for all 7 experiments are shown in Table 90.

The slit sampler observations will be considered first. A total of 2125 cu.ft. of air was examined in the 7 experiments and was found to contain 85 particles carrying Staph.aureus, on average 0.04 per cu.ft.. Some Staph.aureus were found on each day and in each of the rooms examined; the average numbers for the different rooms and days ranged from 0.010 to 0.10 per cu.ft. (see Table 90). About half of the individual 25-cu.ft. samples did not contain any Staph.aureus; the positive samples contained between 1 and 8 particles carrying Staph.aureus.

A total of 15,615 bacterial colonies of all kinds were found on the plates exposed in the slit sampler for examination of the 2125 cu.ft. of air; the average air contamination with "all bacteria" was thus 7.4 bacteria-carrying particles per cu.ft.. The average numbers of "all bacteria" ranged in the different experiments from 1.5 to 14.2 per cu.ft. (see Table 90); the numbers in the different 25-cu.ft. samples ranged from 0.56 to 22.4 per cu.ft. (see Tables 83-89).

Taking/

Taking all experiments together, Staph.aureus comprised 0.54% of "all bacteria" found in the air by use of the slit sampler; in the 7 experiments, the Staph.aureus percentage ranged between 0.14% and 1.1%.

A search was made for correlation between the air infection level and the contemporary circumstances, both with regard to the individual 25-cu.ft. samples and with regard to the average findings for each of the different experiments. Correlation was not found between the level of air contamination with Staph.aureus and the level of air contamination with "all bacteria", or between the amount of air contamination and the amount of ventilation or the amount of activity. Comparison of the average findings for each of the 7 experiments revealed correlation only in the case of one experiment (No.4, Table 90); in this experiment, the levels of air contamination with Staph.aureus and with "all bacteria" were both very low and, appropriately, the amount of ventilation was great and the number of "entrances" small. On the other hand, the greatest air contamination with "all bacteria" occurred on the same occasion as a slight air contamination with Staph.aureus (Experiment No.7, Table 90). The most striking discrepancy was apparent in another experiment (No.5, Table 90), when a very high air infection was associated with conditions which seemed ideal as regards air hygiene; on this day the amount of air contamination with Staph.aureus was the highest encountered on any/

any day and the amount of air contamination with "all bacteria" was greater than the average, yet the number of "entrances" was not very great, bright sunlight was streaming into the room, the doors and windows were wide open and ventilation was obviously very great as evidenced by a perceptible breeze.

In proportion to the recovery rate on the contemporary blood agar settling plates, the slit sampler recovery of Staph.aureus was only very slightly less on MacConkey plates (0.045 per cu.ft. to 0.55 per plate-hour, average for Experiments No.1-6, Table 90) than on nutrient agar plates (0.020 per cu.ft. to 0.20 per plate-hour, Experiment No.7). This suggests that MacConkey medium is as suitable for growth of Staph. aureus as the generally favourable nutrient agar.

The observations made with settling plates exposed beside the slit sampler may best be considered in relation to the average findings summarised in Table 90. The average recovery rate for all 7 experiments was 0.097 of Staph.aureus and 34 of "all bacteria" per 10-sq.in. MacConkey settling plate per hour, and 0.46 of Staph.aureus and 71 of "all bacteria" per 10-sq.in. blood agar settling plate per hour. At first consideration, the difference in recovery rate between the two media suggests that MacConkey's medium grew only 20% of the Staph.aureus and 50% of "all bacteria" which were able to grow on blood agar. However, it can not be concluded that MacConkey's medium is less efficient/

efficient than blood agar for recovery of Staph.aureus, because the media were not tried under strictly comparable conditions; each blood agar plate was exposed for only 25 minutes, while each MacConkey plate was exposed for about 6 hours. The greater crowding on the MacConkey plates, by confluence, dwarfing and suppression of colonies, may have been the main reason for the lower Staph.aureus counts. The Staph.aureus recovery rate on these MacConkey settling plates exposed for 6 hours in the morning (0.097 per plate per hour) was considerably greater than that on the MacConkey settling plates exposed for 24-hour periods (0.035 per plate per hour, see Table 82 and Section 14). The lower rate on the 24-hour plates may have been due in part to the exposure covering the quiet night hours when air infection was minimal, but probably also to the very great colony crowding.

These comparisons suggest that, because of colony crowding, the Staph.aureus counts obtained on the 6-hour and 24-hour settling plates considerably underestimated the number of Staph.aureus particles which actually settled on the plates.

Table 82:- Number of Particles Carrying Coagulase Positive Staph.aureus Settling from Air on to Single 10-Sq.In. Culture Plates Exposed During 24 Hours in Rooms of a Maternity Hospital.

Date.	Nursery A		Nursery B		Mothers' Ward		Wash Room	
	Mc. Plate	SM. Plate	Mc. Plate	SM. Plate	Mc. Plate	SM. Plate	Mc. Plate	SM. Plate
12/3	0	0	0	5	-	-	0	0
14/3	0	0	0	0	-	-	0	0
17/3	2	-	0	-	-	-	0	-
19/3	0	0	1	0	-	-	4	0
21/3	0	0	0	0	-	-	0	0
24/3	0	0	0	0	-	-	1	0
26/3	0	0	0	0	-	-	0	0
28/3	0	1	0	0	-	-	0	1
31/3	0	0	0	1	-	-	0	0
2/4	1	0	0	0	-	-	0	0
6/4	4	-	0	-	-	-	0	-
8/4	0	-	0	0	-	-	1	-
10/4	0	0	2	3	-	-	0	2
14/4	3	1	2	2	-	-	1	3
16/4	1	0	1	1	-	-	0	1
18/4	1	0	1	0	-	-	2	1
21/4	1	0	0	0	-	-	0	-
23/4	0	0	2	0	-	-	2	0
25/4	1	0	1	0	-	-	2	0
28/4	3	0	1	0	-	-	0	3
30/4	0	0	0	1	-	-	3	1
2/5	1	0	0	0	-	-	1	1
5/5	2	1	0	2	-	-	3	-
8/5	0	0	0	1	-	-	-	-
9/5	0	1	0	1	-	-	-	-
12/5	1	0	0	0	0	0	-	-
16/5	0	0	0	3	-	-	-	-
19/5	0	0	0	0	0	0	-	-
23/5	5	-	1	-	1	-	-	-
26/5	0	5	1	0	0	2	-	-
30/5	2	5	3	3	1	1	-	-
6/6	1	-	1	-	0	-	-	-
9/6	1	2	1	3	1	0	-	-
11/6	1	5	1	2	0	2	-	-
13/6	0	3	2	7	1	1	-	-
16/6	2	0	1	1	0	1	-	-
20/6	7	2	0	2	0	2	-	-
23/6	0	2	4	1	3	4	-	-
27/6	2	1	0	1	2	0	-	-
30/6	1	1	0	0	1	0	-	-
Total	43 per 40 days	30 per 35 days	26 per 40 days	40 per 36 days	10 per 14 days	13 per 12 days	20 per 23 days	13 per 18 days
Staph.aureus % of All Colonies from 23/5 to 30/6.	1.3%	1.1%	0.7%	0.8%	0.2%	0.2%	-	-

Mc.:- MacConkey Medium. SM.:- Salt Milk Medium.

Table 83:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air and per 25-Minute 10-Sq.In. Settling Plates at Various Times during Morning of 18/6/47 in 5500 Cu.Ft. Nursery A Containing 26 Babies' Cots Most of Which Were Occupied.

Time	Entrances	Number of Colonies on MacConkey Plate per 25 Cu.Ft. of Air by Slit Sampler		Number of Colonies per 25-Minute 10-Sq. In. Blood Agar Settling Plate	
		Staph. aureus	All Bacteria	Staph. aureus	All Bacteria
07.30-55	15	0	180	0	18
08.00-25	20	0	160	0	26
08.30-55	17	0	140	0	23
09.00-25	7	0	196	1	33
09.30-55	6	0	120	0	6
10.00-25	12	0	200	1	26
10.30-55	5	1	132	1	22
11.00-25	11	0	216	0	19
11.30-55	13	1	204	0	18
12.00-25	14	4	154	1	23
12.30-55	7	0	53	0	14
01.00-25	7	1	48	1	5
01.30-55	2	6	104	0	6
Total:-		13 per 325 cu.ft.	1907 per 325 cu.ft.	5 per 5½ hours	239 per 5½ hours
Average:-		0.040 per cu.ft.	5.9 per cu.ft.	0.9 per hour	43 per hour

07.30-2.00 per Two 10-Sq.In. MacConkey Settling Plates	Staph.aureus:- 1 All Bacteria:- 280	
	Staph.aureus:- 0.077 per plate per hour All Bacteria:- 22 per plate per hour	

Table 84:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air and per 25-Minute 10-Sq.In. Settling Plate at Various Times during Morning of 25/6/47 in 5500 Cu.Ft. Nursery A Containing 26 Babies' Cots Most of Which Were Occupied.

Time	Entrances	Number of Colonies on MacConkey Plate per 25 Cu.Ft. of Air by Slit Sampler		Number of Colonies per 25-Minute 10-Sq. In. Blood Agar Settling Plate	
		Staph. aureus	All Bacteria	Staph. aureus	All Bacteria
08.00-25	4	2	152	0	11
08.30-55	6	3	160	0	15
09.00-25	1	0	160	0	6
09.30-55	5	0	90	0	11
10.00-25	4	0	100	0	12
10.30-55	4	0	86	0	14
11.00-25	2	0	148	0	21
11.30-55	13	1	108	0	7
12.00-25	5	1	132	0	11
12.30-55	9	3	156	1	17
01.00-25	1	1	220	0	10
01.30-55	1	0	60	-	-
Total:-		11 per 300 cu.ft.	1572 per 300 cu.ft.	1 per 4½ hours	135 per 4½ hours
Average:-		0.037 per cu.ft.	5.2 per cu.ft.	0.2 per hour	30 per hour

08.00-2.00 per Two 10-Sq.In. MacConkey Settling Plates	Staph.aureus:- 0 All Bacteria:- 180	
	Staph.aureus:- 0 per plate per hour All Bacteria:- 15 per plate per hour	

Table 85:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air at Various Times during Morning of 14/5/47 in 3700 Cu.Ft. Nursery B Containing 14 Babies' Cots Most of Which Were Occupied.

Time	Number of Colonies on MacConkey Plate per 25 Cubic Feet of Air by Slit Sampler	
	Staph.aureus	All Bacteria
07.30-55	0	207
08.00-25	0	162
08.30-55	0	163
09.00-25	0	151
09.30-55	0	78
10.00-25	0	61
10.30-55	2	91
11.00-25	0	130
11.30-55	0	192
12.00-25	0	113
12.30-55	5	85
01.00-25	1	101
Total:-	8 per 300 cu.ft.	1534 per 300 cu.ft.
Average:-	0.027 per cu.ft.	5.1 per cu.ft.

Table 86:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air and per 25-Minute 10-Sq.In. Settling Plate at Various Times during Morning of 21/5/47 in 3700 Cu.Ft. Nursery B Containing 14 Babies' Cots Most of Which Were Occupied.

Time	Entrances	Number of Colonies on MacConkey Plate per 25 Cu.Ft. of Air by Slit Sampler		Number of Colonies per 25-Minute 10-Sq. In. Blood Agar Settling Plate	
		Staph. aureus	All Bacteria	Staph. aureus	All Bacteria
08.00-25	4	2	68	0	12
08.30-55	7	0	49	0	8
09.00-25	10	0	46	0	8
09.30-55	2	1	45	0	6
10.00-25	5	0	44	0	9
10.30-55	3	0	35	1	12
11.00-25	2	0	33	0	16
11.30-55	8	0	60	0	8
12.00-25	2	0	14	0	8
12.30-55	2	0	18	0	5
1.00-25	3	0	16	0	7
1.30-55	3	0	22	0	8
Total:-		3 per 300 cu.ft.	450 per 300 cu.ft.	1 per 5 hours	107 per 5 hours
Average:-		0.01 per cu.ft.	1.5 per cu.ft.	0.2 per hour	21 per hour

08.00-2.00 per Two 10-Sq.In. MacConkey Settling Plates	Staph.aureus:- 1 All Bacteria:- 81	
	Staph.aureus:- 0.08 per plate per hour All Bacteria:- 6.8 per plate per hour	

Table 87:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air and per 25-Minute 10-Sq.In. Settling Plate at Various Times during Morning of 28/5/47 in 5400 Cu.Ft. Mothers' Ward Containing 9 Beds All of Which Were Occupied.

Time	Entrances	Number of Colonies on MacConkey Plate per 25 Cu.Ft. of Air by Slit Sampler		Number of Colonies per 25-Minute 10-Sq. In. Blood Agar Settling Plate	
		Staph. aureus	All Bacteria	Staph. aureus	All Bacteria
07.30-55	5	8	539	1	96
08.00-25	14	2	478	0	67
08.30-55	11	5	496	-	-
09.00-25	3	1	146	0	36
09.30-55	12	1	127	0	43
10.00-25	4	1	158	0	38
10.30-55	3	3	144	0	23
11.00-25	6	3	123	1	62
11.30-55	3	1	242	0	45
12.00-25	7	0	119	2	38
12.30-55	14	1	111	0	24
01.00-25	15	0	128	0	27
01.30-55	3	7	173	1	51
Total:-		33 per 325 cu.ft.	2984 per 325 cu.ft.	5 per 5 hours	550 per 5 hours
Average:-		0.10 per cu.ft.	9.2 per cu.ft.	1 per hour	110 per hour

07.30-2.00 per One 10-Sq.In. MacConkey Settling Plate	Staph.aureus:- 1 All Bacteria:- 440	
	Staph.aureus:- 0.15 per plate per hour All Bacteria:- 68 per plate per hour	

Table 88:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air and per 25-Minute 10-Sq.In. Settling Plate at Various Times during Morning of 4/6/47 in 5400 Cu.Ft. Mothers' Ward Containing 9 Beds All of Which Were Occupied.

Time	Entrances	Number of Colonies on MacConkey Plate per 25 Cu.Ft. of Air by Slit Sampler		Number of Colonies per 25-Minute 10-Sq. In. Blood Agar Settling Plate	
		Staph. aureus	All Bacteria	Staph. aureus	All Bacteria
08.30-55	13	0	362	0	55
09.00-25	0	1	158	0	8
09.30-55	3	0	380	0	81
10.00-25	7	4	435	0	61
10.30-55	5	1	314	0	33
11.00-25	7	0	246	0	31
11.30-55	9	1	114	1	26
12.00-25	13	1	93	0	25
12.30-55	10	1	92	0	24
01.00-25	9	1	432	-	-
01.30-55	4	1	292	0	26
Total:-		11 per 275 cu.ft.	2918 per 275 cu.ft.	1 per 4½ hours.	370 per 4½ hours
Average:-		0.040 per cu.ft.	10.6 per cu.ft.	0.24 per hour	87 per hour

08.30-2.00 per Two 10-Sq.In. MacConkey Settling Plates	Staph.aureus:- 2 All Bacteria:- 664	
	Staph.aureus:- 0.18 per plate per hour All Bacteria:- 60 per plate per hour	

Table 89:- Number of Coagulase-Positive Staph.aureus-Carrying Particles and All Bacteria-Carrying Particles per 25 Cu.Ft. of Air and per 25-Minute 10-Sq.In. Settling Plate at Various Times during Morning of 2/7/47 in 5400 Cu.Ft. Mothers' Ward Containing 9 Beds 7 of Which Were Occupied.

Time	Entrances	Number of Colonies on Nutrient Agar Plate per 25 Cu.Ft. of Air by Slit Sampler		Number of Colonies per 25-Minute 10-Sq. In. Blood Agar Settling Plate	
		Staph. aureus	All Bacteria	Staph. aureus	All Bacteria
07.30-55	3	3	360	1	35
08.00-25	24	0	400	0	170
08.30-55	2	-	-	-	-
09.00-25	12	0	320	0	84
09.30-55	2	1	560	0	100
10.00-25	15	0	400	0	80
10.30-55	4	0	240	0	24
11.00-25	2	1	200	0	15
11.30-55	4	1	280	0	28
12.00-25	9	0	280	0	30
12.30-55	21	0	380	0	27
01.00-25	12	0	560	0	39
01.30-55	4	0	270	0	30
Total:-		6 per 300 cu.ft.	4250 per 300 cu.ft.	1 per 5 hours	662 per 5 hours
Average:-		0.020 per cu.ft.	14.2 per cu.ft.	0.2 per hour	132 per hour

Table 90:- Air Contamination with Staph.aureus and other Bacteria in Rooms of Maternity Hospital: Summary of Average Findings in 7 Experiments Recorded in Tables - .

Experiment Number:-		1	2	3	4	5	6	7*	
Room:-		Nursery A	Nursery A	Nursery B	Nursery B	Mothers' Ward	Mothers' Ward	Mothers' Ward	Overall Average
Room Volume in Cubic Feet:-		5500	5500	3700	3700	5400	5400	5400	
Floor Area in Square Feet:-		420	420	288	288	416	416	416	
Date:-		18/6/47	25/6/47	14/5/47	21/5/47	28/5/47	4/6/47	2/7/47	
MacConkey Plates in Slit Sampler	Staph.aureus per Cu.Ft.:-	0.040	0.037	0.027	0.010	0.10	0.040	0.020 *	0.039
	"All Bacteria" per Cu.Ft.:-	5.9	5.2	5.1	1.5	9.2	10.6	14.2	7.4
	Staph.aureus % of All Bact:-	0.68%	0.71%	0.53%	0.67%	1.1%	0.38%	0.14%	0.60%
10-Sq.In. MacConkey Settling Plates	Staph.aureus per Plate-Hour:	0.077	0	-	0.080	0.15	0.18	-	0.097
	"All Bacteria" p.Plate-Hour:	22	15	-	7	68	60	-	34
	Staph.aureus % of All Bact:	0.35%	0%	-	1.2%	0.23%	0.30%	-	0.42%
10-Sq.In. Blood Agar Settling Plates	Staph.aureus per Plate-Hour:	0.9	0.2	-	0.2	1.0	0.24	0.2	0.46
	"All Bacteria" p.Plate-Hour:	43	30	-	21	110	87	132	71
	Staph.aureus % of All Bact:	2.1%	0.67%	-	0.95%	0.91%	0.28%	0.15%	0.84%
Entrances:-		136	55	-	51	100	80	114	
Ventilation:-		Medium	Medium	Medium	Great	V.Great	Great	Medium	

* Nutrient agar plates in slit sampler in experiment 7.

Section 17: Amount of air contamination with Staph. aureus and size of inhalation dose.

The observations recorded above give a measure of Staph.aureus contamination of the air in the rooms of the maternity hospital. The hospital air was infected with Staph.aureus more or less constantly throughout the 16 weeks of the investigation. Similar small numbers of Staph.aureus were found settling from the air of one or more rooms, on 43 out of 46 days when observations were made. The amount of Staph.aureus air contamination was similar in each of the four rooms examined; namely, two nurseries, a mothers' ward and a wash room.

The observations made with 24-hour settling plates showed an average deposition rate of Staph.aureus particles per 10 sq.in. area per 24 hours of 1.2 in Nursery A, of 0.75 in Nursery B, and of 0.2 in the Mothers' Ward. For reasons already discussed (Section 15), these figures probably underestimate the true deposition rate. Thus, on average in each 24 hours, more than 7100 Staph.aureus particles fell on the 420 sq.ft. floor area of Nursery A, more than 3100 Staph.aureus particles fell on the 288 sq.ft. floor area of Nursery B, and more than 1100 Staph.aureus particles fell on the 416 sq.ft. floor area of the Mothers' Ward. The number of particles falling out of the air represents the number put into the air less the number lost by ventilation. It may be concluded that/

that the average daily rate at which Staph.aureus-carrying particles were liberated into the air, was more than 7100 per day (300 per hour) in Nursery A, more than 3100 per day (130 per hour) in Nursery B, and more than 1100 per day (45 per hour) in the Mothers' Ward. These calculated rates are probably much lower than the actual rates because of incomplete counts being obtained from the crowded 24-hour settling plates. Staph.aureus deposition rates calculated from the counts on the 25-minute blood agar settling plates, were much higher and probably more accurate. These indicated that during the morning Staph.aureus particles were liberated into the air at a rate of more than 3300 per hour in Nursery A, at a rate of more than 830 per hour in Nursery B, and at a rate of more than 3000 per hour in the Mothers' Ward.

The slit sampler observations showed that the average daytime air contamination in rooms of the maternity unit was 0.040 Staph.aureus-carrying particles per cu.ft. (i.e. 200 in a 5000 cu.ft. room). Assuming that there was not any Staph.aureus contamination of the air during 10 hours at night, an adult respiring some 500 cu.ft. of air per day would inhale during each day of residence in the hospital, on average, 12 particles carrying Staph.aureus; he would inhale about 1 Staph. aureus particle per hour during the daytime. The new born child respiring 44 cu.ft. of air per day would inhale, on average, 1 Staph.aureus particle per day.

The/

The adult's daily respiration rate of 500 cu.ft. per day was calculated for a respiration frequency of 20 per minute and a tidal air of 500 c.c.. The new born child's daily respiration rate of 44 cu.ft. per day was calculated for a respiration frequency of 44 per minute and a tidal air of 19.0 c.c. (data from Nelson, 1946).

If it is supposed that each infected particle carries between 1 and 10,000 Staph.aureus cells, this may be regarded as the average daily inhalation dose of the new born babies during their stay in the hospital.

On average for the whole investigation, particles carrying Staph.aureus comprised 0.54% of all bacteria-carrying particles found in the air by use of the slit sampler with MacConkey plates, 0.84% of those settling from the air and growing on blood agar plates, and 0.7% of those settling from the air and growing on MacConkey plates. It is concluded that Staph.aureus comprised about ½% of all the bacteria in the air of the maternity hospital.

Positive information about the causes of air infection was not obtained in attempts to correlate the amount of air infection with the contemporary circumstances. It was noted that ventilation did not play a major part in determining the level of air infection; the very heaviest air infection was observed in the Mothers' Ward on a day when the doors and windows were wide open, and a breeze was perceptible within the room.

Section 18: Role of airborne Staph.aureus in spread of the infections.

Previous investigations of the spread of Staph.aureus infections among mothers and babies in maternity hospitals have revealed that such hospitals contain many sources and reservoirs of Staph.aureus and that the pattern of contagion may be fairly complex (Duncan and Walker, 1942; Allison and Hobbs, 1947). Productive sources of Staph.aureus include the open infected lesions in babies (e.g. conjunctivitis and septic skin lesions), the breast milk of mothers' with mastitis, the nose or throat of the usually numerous "healthy carriers" among the babies, mothers, nurses, other attendants and visitors (the Staph.aureus nasal carriage rate often exceeds 50%), and the breast milk of a large proportion of mothers with apparently normal breasts. Among reservoirs and vehicles of Staph.aureus are hands, clothing, blankets, floor-dust and the air.

There is no general agreement as to what is the main and usual pattern of contagion, whether from mother to baby, nurse to baby, baby to baby, baby to mother or baby to nurse, or whether spread occurs in all ways indiscriminately. Duncan and Walker (1942) found that the Staph.aureus strain in a mother's milk was almost always of the same serological type as the Staph.aureus strain in her baby's throat, but usually of a different type from the Staph.aureus, if any, in her own throat; these authors concluded that the babies were/

were infected mainly from other babies in the common nursery ward and occasionally from the nose or throat of nurse or mother, and that the infected baby caused infection of its mother's breast in suckling.

Allison and Hobbs (1947) concluded from the widespread occurrence of Staph.aureus carriage in the nursing staff and also from observations of ward practice, that the babies were infected in the common nursery and not from their mothers. The observations suggested that the main source of infection was the nasal passages of the nurses and that infection was transferred to the babies probably via the hands; infection from baby to baby by indirect contact through fomites, communal bath and towel, was thought to play a part. Because the numbers of Staph.aureus in the air appeared to be small, Allison and Hobbs did not regard airborne infection as playing an important role in the pattern of contagion.

The main habitat of Staph.aureus is the anterior nares; nasal "carriage" is commoner than parasitisation of any other part of the body. The nose appears to be the most favourable site for reception and growth of Staph.aureus. This suggests that the usual course of infection is for a few Staph.aureus from an environmental source to gain access in the first place to the nose of the patient (baby), to reproduce in the nose until large numbers are present, and then, as opportunity offers, to spread to any other part of the body having

low/

low resistance and there produce an infective lesion, for instance through a skin abrasion to produce a pemphigous bulla as described by Knott and Blaikley (1944). This view is supported by the finding that babies with Staph.aureus infections of the skin usually yield Staph.aureus of the same type in swabs taken from the nose; for instance, on examining 66 babies with pemphigus neonatorum, Allison and Hobbs (1947) found that culture of the skin lesion yielded Staph.aureus in all 66 cases (of the epidemic type in 61 cases), and that culture from the nose yielded Staph.aureus in 60 cases (of the corresponding epidemic type in 37 cases). It is possible, of course, that the nasal infection in these cases was secondary to the skin infection. The absence of "nasal carriage" of Staph.aureus in some of the babies having Staph.aureus infection of the skin, indicates that sometimes the skin may be infected directly with Staph.aureus received from the environment.

Infection of a baby follows reception into its nose, into its mouth or on to its skin, of Staph.aureus from the nose, throat or infective lesion of nurse, mother or another baby. The two likely modes of transmission are, firstly, airborne infection by Staph. aureus carried in dust particles (or possibly droplet nuclei) which are inhaled directly into the nose or throat, and, secondly, contact infection by Staph.aureus carried on the nurse's fingers, on towels, on utensils, in/

in bath water, or on the feeding bottle teat, and introduced into the baby's mouth or nose, either directly or by the baby's own fingers.

The main interest of the present investigation is that a measure has been obtained of the frequency and amount of Staph.aureus contamination of the air in a maternity hospital. This constitutes an advance towards elucidation of the pattern of contagion and towards evaluation of the role of airborne infection. Unfortunately, there is not yet available adequate information about the number of Staph.aureus which are transmitted by contact, to make possible by comparison a definite assessment of the relative importance of airborne infection and contact infection.

The settling plate observations made in the present investigation showed that Staph.aureus particles were falling from the air of the nurseries on to the skin and other exposed surfaces of the babies at the rate of 1 to 10 per day per 10 sq.in.. The slit sampler observations showed that each baby was inhaling each day, on average, 1 infected particle which carried between 1 and, perhaps, 10,000 Staph.aureus cells. If such a small dose, 1 infected particle per day, is not sufficient to initiate infection or commensal colonisation, then airborne infection can not have been of any importance. On the other hand, if one infected particle is sufficient to initiate infection or commensal colonisation, airborne infection must surely have/

have played a most important part in spreading disease in the maternity unit, since all babies must have inhaled an infected particle within a day or so after birth; the air of the hospital contained sufficient Staph.aureus to ensure universal infection of the babies, the mothers and the nurses. If this latter view is accepted, it must be assumed that any inmates of the hospital remaining free of Staph.aureus infection and "carriage", such as the babies and nurses yielding negative nasal swabs, were uninfected because they had resisted infection; none could have avoided encountering and inhaling Staph.aureus.

There are various reasons for accepting this view that a single Staph.aureus-carrying particle may suffice to initiate nasal colonisation in susceptible persons, especially babies, and that airborne infection was therefore of great importance. There does not appear to be any other mode of transmission which would introduce much larger numbers of Staph.aureus into the infant's nose, and which would do this so frequently as to account for the very high incidence of nasal infections (50% on July 1st). Occasionally, under special circumstances, very large numbers of Staph.aureus may be put into the baby's mouth or nose by contact: as when the nurse's finger, liberally smeared with infected exudate from her nose or from the lesion of another baby, is without washing put into the baby's mouth or allowed to touch the feeding bottle teat.

However/

However, such a gross breach of nursing technique can not have happened with the very high frequency necessary to account for the incidence of infection. The operation of a more ready mechanism of transmission seems indicated. The more indirect modes of contact infection must have occurred frequently, but it is not likely that large numbers of Staph.aureus cells were transferred. The experiments recorded in Part 4 showed that only small numbers of Staph.aureus colonies (e.g. 1 to 100) developed on culture plates which were heavily handled by nasal carriers. Thus, in the absence of gross smearing of the fingers with infected exudate, only a few Staph.aureus would be transferred to a baby's mouth on introduction of the finger of a nurse who was a nasal carrier.

For these reasons, it is concluded that airborne Staph.aureus played an important part in the causation of infections in the maternity unit.

Section 19: Comparison of the different culture media used for isolation of Staph.aureus from the air.

It must be stated that this aspect of the present investigation was not satisfactory. Analysis of the results showed that these did not supply an adequate basis for conclusions as to the relative value of nutrient agar, blood agar, MacConkey's medium and salt-milk medium for the isolation of Staph.aureus from air. The observations made with 24-hour settling plates (Section 14 and Table 82) showed the same Staph.aureus recovery rate per plate-hour and the same Staph.aureus percentage of all colonies, when MacConkey medium was used as when the salt-milk medium was used. However, most of these 24-hour plates were overcrowded and this may have altered the selective properties of the media. Comparison between the slit sampler recovery rate on nutrient agar and that on MacConkey medium, suggested that these two media were equally effective for isolation of Staph.aureus (Section 15); however, the comparison was based on only a single experiment with nutrient agar plates, so that much significance can not be attached to the findings. Comparison between the settling plate recovery rate on 6-hour MacConkey plates and that on 25-minute blood agar plates, suggested that MacConkey's medium grew only 20% of the Staph.aureus which grew on blood agar; however, the poorer recovery on the MacConkey plates is thought to have been due more to the overcrowding which resulted from the longer exposure than to the nature of the medium (see Section 15).

Part 5c

Bacterial Content of Air in a Fever Hospital Ward Occupied by Infants with Intestinal Infection with Salm.typhimurium.

Section 20: Account of ward and incidence of infection.

Observations were made in a ward of an Infectious Fevers Hospital. The ward was large and well ventilated; all of the several windows on each side of it were partly open. There were 8 babies present in the ward, and not any other patients; the babies' cots were spaced well apart. Of the 8 babies, 7 had intestinal infection with Salm.typhimurium as result of a ward epidemic.

Section 21: Experimental methods.

The air was examined with a sieve sampler as described in Part 6. This sampler was operated at a sieve-agar distance of 2 millimeters and at an air intake rate of 19 cu.ft. per minute. The observer wore a sterile dust-proof suit while making the observations.

The culture medium used for isolation of Salm.typhimurium was the desoxycholate-citrate agar of Leifson as modified by Hynes. The very high selectivity of this medium for Salmonella organisms afforded good hope for successful isolation of the pathogenic bacterium in this outbreak.

Some air samples were taken on to blood agar for measurement of the total bacterial content of the air, and some samples were taken on to the alkaline tellurite medium of Ludlam (1949) for isolation of Staph.aureus.

Section 22: Bacterial content of the air.

Observations were made in the ward on March 24th, 1948, between 11.30 a.m. and 12.30 p.m.. During this mid-day hour, two doctors and the ward sister were on their "ward round", examining babies and disturbing the bedclothes to do so; nurses were busy changing and feeding the babies.

In all, 8 desoxycholate-citrate plates were exposed in the sieve sampler; 20 cu.ft. samples of air were taken on to 7 plates and a 60 cu.ft. sample on to 1 plate, making a total of 200 cu.ft. of air examined. Of the 8 samples, 3 samples were taken with the sampler in the centre of the ward, 4 samples were taken with the sampler held immediately over the cots of babies who were being examined, and 1 sample was taken with the sampler held just beside a seated nurse who was holding and feeding a baby. Salm.typhimurium was not found in the 200 cu.ft. of air. On the 8 desoxycholate-citrate plates, there were only 9 colonies of all kinds, and none of these was a Salmonella colony.

Two further desoxycholate-citrate plates were used for sampling, respectively, a roller towel which was used by persons washing their hands after examining or attending the babies, and the front of the gown of a nurse who had just been bathing, changing and feeding babies. The samples were taken by "vacuuming" the cloth surface with the sieve sampler. Salm.typhimurium was cultured from both the towel and the nurse's gown.

Two/

Two blood agar plates were exposed in the sieve sampler in the centre of the ward; 20 cu.ft. samples of air were taken on to each plate. The first plate yielded 136 colonies of saprophytic bacteria (7 per cu. ft.) and the second plate yielded 304 colonies (15 per cu.ft.).

Three plates of Ludlam's medium were exposed in the sieve sampler; two samples of 20 cu.ft. each were taken with the sampler held just above babies who were being fed and one sample of 60 cu.ft. was taken with the sampler in the centre of the ward at the end of the "ward round". On these 3 plates corresponding to 100 cu.ft. of air, there were 2 colonies of coagulase-positive Staph.aureus (1 on the 60 cu.ft. plate) and 558 colonies of other bacteria; that is, there were 0.02 Staph.aureus per cu.ft. and 5.6 "other bacteria" per cu.ft..

Section 23: Discussion and conclusions.

Gram-negative bacteria are less resistant to light and drying than are the Gram-positive bacteria. For this reason, pathogenic intestinal Gram-negative bacteria seldom have been found in the air.

The present ward epidemic seemed to offer the most favourable conditions for air infection with a pathogenic intestinal Gram-negative bacterium. The air samples were taken at a time when the babies, their clothing and their bed-clothes were being disturbed; many of the air samples were taken in the immediate vicinity of the babies while these dust-raising activities were in progress. It was thought that at least slight air infection with Salm.typhimurium might be produced by liberation of dust from clothing which had been subjected to faecal contamination.

However, in spite of the presence of many infected babies and in spite of the sampling of a large volume of air, Salm.typhimurium was not found in the air. This may be taken as a confirmation of the generally accepted view that Salmonella infections are not airborne.

The finding of Salm.typhimurium on the ward towel and on a nurse's gown, accords with the belief that the Salmonella infections are commonly spread by contact with such objects, especially when soiled with excreta and damp.

Section 24: SUMMARY OF PART 5.

Part 5a: Observations were made with a slit sampler of the bacterial content of the air in rooms of a residential training institution during an epidemic of Strept.pyogenes throat infections. The institution housed 765 young men. The epidemic occurred in October and November, 1942; 58 cases of tonsillitis, 29 cases of scarlet fever and a high incidence of "healthy carriers" were reported between October 11th and November 21st. The observations were made at the height of the epidemic between November 13th and 29th.

A total of 875 cubic feet of air, in 150 samples, was examined and found to contain 178 particles carrying Strept.pyogenes, that is 0.2 per cu.ft.. Strept.pyogenes-carrying particles comprised only 0.5% of all bacteria-carrying particles in the air, as revealed by counts of colonies on ordinary blood agar plates.

From the various measurements of air infection, it was calculated that each inmate of the institution at the height of the epidemic must have inhaled every day, on average, about 100 particles carrying Strept.-pyogenes. The significance of this inhalation dose has been discussed and reasons have been given for believing that it would be sufficient to cause infection in many persons. It is concluded that airborne infection played an important part in the spread of the epidemic.

The/

The average amounts of air infection in a dormitory, cinema hall, schoolroom and recreation room were, respectively, 0.22, 0.33, 0.63 and 0.38 Strept.-pyogenes-carrying particles per cu.ft., when these rooms were occupied to the extent of 0.8, 3.8, 1.9 and 2.3 persons, on average, per 1000 cu.ft. of room space.

The amount of air infection with Strept.pyogenes and with "all bacteria" was large only during periods when the rooms were occupied; after the occupants had departed and the rooms were left unoccupied, the air infection diminished rapidly to reach a very low level within 30 minutes (e.g. 10% of the level "during occupation"). Air infection depended not only upon occupation, but also upon the movements and activities of the occupants; there was very little infection of the air in the dormitory at night when the occupants were all present, but were asleep in bed. The amount of air infection in the different rooms was not proportional to the degree of crowding; apparently, the amount of air infection depended less on the number of persons present than on the amount and kind of their activities. The influence of ventilation on the amount of air infection appeared to be very slight; air contamination with Strept.pyogenes and other bacteria was about as great in the very well ventilated dormitory as in the very poorly ventilated cinema hall and recreation room.

Of the 150 air samples, 39 samples comprising 133 cu.ft./

133 cu.ft. of air were taken on to ordinary blood agar plates, and 111 samples comprising 742 cu.ft. of air were taken on to plates of blood agar incorporating crystal violet in a concentration of 1 in 1000,000. The overall average number of Strept.pyogenes recovered per cu.ft. of air was the same on the crystal violet blood agar as on the ordinary blood agar; apparently, none of the Strept.pyogenes were suppressed and missed because of the crystal violet. On the other hand, the crystal violet suppressed growth of all but 5% of the other kinds of airborne bacteria which grew on the ordinary blood agar. Strept.pyogenes comprised 0.5% of all colonies on ordinary blood agar, but 11.1% of all colonies on the crystal violet blood agar. Thus, the crystal violet blood agar proved very satisfactory as a selective medium for cultivation of Strept.pyogenes from the air; its inhibition of other bacteria was such as would allow a 20-fold increase in the volume of air sampled per plate, as compared with ordinary blood agar, without danger of colony overcrowding.

Part 5b: During a 16-week period from March 12th to July 2nd, 1947, observations were made of the bacterial content of the air in rooms of a maternity hospital. Many cases of Staph.aureus infection occurred both before and during the period of investigation; the Staph.aureus infections included conjunctivitis, septic spots on the skin and pneumonia among the babies, and boils/

boils, breast abscess, Caesarian wound sepsis and vaginal infection among the mothers. There were also many healthy carriers of Staph.aureus; on one occasion (July 1st), 2 out of 7 nurses and 11 out of 26 babies yielded Staph.aureus on swabs taken from the nose.

Observations were made in four rooms, a 5500 cu.ft. nursery (A) which contained 26 babies' cots, a 3700 cu. ft. nursery (B) which contained 14 babies' cots, a 5400 cu.ft. ward for mothers with 9 beds, and a 1000 cu.ft. wash room which was entered by nurses to wash linen and utensils. The space per person, the ventilation and the cleanliness of the rooms was judged very good.

On each of 40 days at intervals between March 12th and July 2nd, observations were made by simple exposure of 10-sq.in. culture plates for 24 hours in both nurseries and in either the mothers' ward or the wash room. Small numbers of Staph.aureus particles, from 1 to 7, fell on to these 24-hour "settling plates" in at least one room on 36 of the 40 days; similar numbers of Staph.aureus were found at different times throughout the period of investigation, and similar numbers in each of the four rooms. The overall average rate of deposition observed was 0.037 Staph.-aureus particles per 10-sq.in. plate per hour; however, it is thought that many Staph.aureus were missed because of colony overcrowding on the 24-hour settling plates.

Observations/

Observations were made with 25-minute settling plates and with a slit sampler throughout a morning period of 6 hours, on two days in Nursery A, on two days in Nursery B and on three days in the Mothers' Ward. Small numbers of Staph.aureus were found in the air of each room on each day. The 25-minute settling plates showed an average deposition rate of 0.46 Staph.aureus particles per 10-sq.in. plate per hour; Staph.aureus must have fallen at about this rate on the exposed skin surface of the babies, perhaps one or two infected particles landing on each baby's face per day.

In the three rooms and on the seven days, a total of 2125 cubic feet of air was examined with the slit sampler and was found to contain 85 particles carrying Staph.aureus, that is 0.040 per cu.ft.; the average numbers for the different days ranged from 0.01 to 0.10 per cu.ft.. It was calculated from these measurements that an adult inmate of the hospital at that time must have inhaled, on average, about 12 Staph.aureus particles per day, and a baby about 1 Staph.aureus particle per day. The significance of this small inhalation dose has been discussed. It is thought to be sufficient to initiate infection in many persons; thus, airborne Staph.aureus probably played an important part in the spread of infections in the hospital. Staph.aureus-carrying particles were found to comprise about ½% of all bacteria-carrying particles present in the air.

It appeared that ventilation did not play a major part in determining the level of air infection; the heaviest air infection occurred on the day when the ventilation was greatest.

Four culture media were used in sampling, nutrient agar, blood agar, MacConkey's medium and salt-milk medium; the latter two were thought to be selective for Staph.aureus, but, although the results obtained did not permit of a conclusive comparison, they did not appear to confer any appreciable advantage over the non-selective media.

Part 5c: Observations were made with a sieve sampler of the bacterial content of the air in an Infectious Fevers Hospital ward which was occupied by 8 babies of whom 7 had intestinal infection with Salm.typhimurium; the air samples were taken during an hour at mid-day while the babies were being examined, changed and fed. Using 8 desoxycholate-citrate agar plates, a total of 200 cubic feet of air was examined; Salm.typhimurium was not found in the air. This finding accords with the general belief that Salmonella infections are not airborne. Cultures from the ward roller towel and from a nurse's gown both yielded a growth of the Salmonella.

Using 3 plates of Ludlam's medium, a total of 100 cubic feet of air was examined and found to contain 2 particles carrying Staph.aureus and 558 particles carrying bacteria of other kinds.

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Part 6

DEVELOPMENT OF A MODIFIED "SIEVE SAMPLER" FOR RAPID BACTERIOLOGICAL EXAMINATION OF LARGE VOLUMES OF AIR.

CONTENTS

Section 1: Introduction.

Many different instruments have been designed for bacteriological examination of air. By far the best is the "slit sampler" described by Bourdillon, Lidwell and Thomas (1941). This sampler operates by impingement of a jet of air on to a collecting surface. Air sucked from the outside at the rate of 1 cu.ft. per minute, passes through a slit intake and impinges on the surface of an agar-containing Petri dish (3½ in. diam.). The dish is situated so that its radius lies parallel to the slit and its agar surface at 2 millimeters from the slit; it is rotated to secure uniform distribution of bacteria. The rate of air intake is regulated by adjustment of a "leak" in the connection to the suction pump; by this, a certain constant pressure difference, observed by a manometer, is maintained across the slit intake. The slit sampler has a high collection efficiency, recovering from the air more than 94% of even the smallest bacteria-carrying particles.

A slit sampler was used in carrying out the investigations which already have been described (Parts 2-5); it was found to be an excellent instrument for most purposes. For some special purposes, however, need was felt for a sampler of another kind. In particular, for "field observations" (i.e. in hospitals, schools and other places outside the laboratory) a sampler was desired which was cheaper,/

cheaper, simpler in operation, more portable, and more rapid in sampling large volumes of air.

The slit sampler is expensive to buy; a simple model is advertised by one manufacturer at £100 and by another at £40. The weight and bulk of the slit sampler and its accessories, including suction pump, pump motor, plate-rotating motor and Petri dishes, is just not too great to make impossible carriage by a single person; however, it is a laborious and difficult task for one person, without help, to carry, instal, operate and remove the slit sampler. The delicacy of some of the fitments, the manometer and plate-distance indicator, necessitate care in handling, a container for its transport and, preferably, a trolley for moving it within the premises being examined. Operation of the slit sampler requires some little care and experience, and can not safely be delegated to partly trained assistants; apart from the loading and unloading of culture plates with aseptic precautions, adjustment of the slit-plate distance, sealing of the door and adjustment of the air flow must be carried out correctly.

The limitation of the ordinary slit sampler to an air-intake rate of 1 cu.ft. of air per minute is often found to be a disadvantage. Usually when making field observations, the time available for observation is limited (e.g. by the duration of the occurrence being observed, as "time of bed-making", "meal-time", etc.). Thus/

Thus, the volume of air sampled depends on the rate of sampling. If this is small, difficulty may be met in demonstrating and counting the pathogenic bacteria in the air, since these are usually very scanty. The advantages in this respect of a rapid intake sampler are very great. Furthermore, for special kinds of experimental work in the laboratory, a rapid-intake sampler is required; for instance, in measuring the amount of air infection produced by a given momentary action (as by a sneeze; see Part 4d), it is necessary, if the air infection is little in amount and rapidly disappearing, to sample as large as possible a proportion of the exposed air in as short as possible a time following the action. Dr. O.M. Lidwell (unpublished; personal communication) has designed a modified slit sampler which, by making use of a 6-in. diameter dish, samples 20 cu.ft. of air per minute instead of 1 cu.ft. per minute. Although use of such a large dish gives better separation of colonies, it entails certain inconveniences, especially at the present time when it is very difficult to procure dishes of other than the standard size (3½ in. diam.).

To meet these special requirements, a sampler was developed by modification of the "sieve-plate sampler" design of Du Buy and Crisp (1944). The sampler of Du Buy and Crisp in many respects resembles the Bourdillon slit sampler; it operates by impingement of air jets on to solid culture medium and thus has a high collection/

collection efficiency; the air intake rate is about 1 cu.ft. per minute; the intake-plate distance is observed by a special indicator. The sieve sampler differs from the slit sampler with regard to its air intake orifice. Instead of being a single slit, the air intake consists of 300 small holes. The holes are distributed uniformly in a plate with area rather less than that of the Petri dish; this arrangement gives a fairly uniform distribution of the bacteria on the surface of the culture medium without rotation of the Petri dish as in the slit sampler. The motor and mechanism used in the slit sampler to effect rotation are dispensed with in the sieve sampler; for a "field sampler", the consequent advantage in portability outweighs the disadvantage of the rather less even distribution of bacteria.

Certain modifications were made in the design of Du Buy and Crisp (1944). Firstly, the sieve-agar distance indicator and the mechanism for adjusting this distance were omitted. A sieve-agar distance of about 2 millimeters (2-2½ mm.) was achieved with regularity by the pouring of all culture plates to a constant depth. Thus, construction and operation of the sampler were both simplified.

The second modification also simplified construction, but was directed primarily to a more important end, namely to increasing the air intake rate from 1 to 20 cu.ft. per minute. A small-sized vacuum cleaner/

cleaner was used as the suction pump. This could readily suck 20 cu.ft. of air per minute through the 300 air-intake holes which had a combined cross-section area of 1.5 sq.cm.. However, in the original design of Du Buy and Crisp the suction pump was connected to the sampler chamber by a tube of only 0.13 sq.cm. in cross-section; this did not allow an air flow approaching 20 cu.ft. per minute. To obviate this restriction, and also to increase portability, the sampler unit was built into the front of the vacuum cleaner, as one unit instead of two, so that there was free and ample communication for the air escaping round the edges of the culture plate to pass back into the pump. The electric motor and pump were found to give a fairly constant rate of air intake, so that it was possible to dispense with means for observing and controlling this.

This modified sieve sampler was found to fulfil all the important requirements of a "field sampler". Its only defects as compared with the slit sampler were lower collection efficiency, lesser accuracy and less even distribution of colonies on the culture plates. On the other hand, it was much cheaper than the slit sampler; £10 was the average cost of three sieve samplers made by a commercial firm, the cost of labour, materials and a second-hand vacuum cleaner being included in this figure. The sieve sampler was much lighter, more robust, more easily carried about and

more/

more easily operated than the slit sampler. Its portability was increased by the provision within the vacuum cleaner cylinder of space for carrying up to about a dozen spare culture plates, which therefore did not require to be carried in a separate package. Most important of all, the modified sieve sampler dealt with air at the rate of about 20 cu.ft. per minute, using standard 3½ inch diameter culture plates.

Design and calibration of the modified sieve sampler was carried out in collaboration with Dr. A.T. Wallace.

Section 2: Construction and Use of Sieve Sampler.

Three sieve samplers were made (No.s 1, 2 and 3). Sieve sampler No.1 was built into a small-sized cylinder-type vacuum cleaner (Electrolux, Series 524, No. 38,960). Sieve samplers No.2 and No.3 were built at different times into another small vacuum cleaner (Electrolux, Model 1144, No. E 44,046; Volt. 230-250 and Watt. 240; A.C.-D.C.).

The sieve plate of sampler No.1 was made of perspex. The sieve plates of samplers No.s 2 and 3 were made of brass. Both materials were found to be perfectly suitable.

The design and construction of sieve sampler No.3 is shown in Figures 21, 22, 23, 24 and 25; the other models were very similar.

The overall design is best appreciated from Figures 21 and 22. The sampler weighed 11 pounds. Its dimensions were 17 in. long x 7 in. high x 6 in. wide. The vacuum cleaner cylinder had been shortened to secure this small size which was desired for the sake of portability. The dust bag of the vacuum cleaner had been discarded. An air deflector was attached to the outlet at the rear end, as shown in Figure 22; this threw the exhaust air-stream upwards and thus prevented raising of dust from the surface on which the sampler was lying.

The final and main alteration to the vacuum cleaner was the mounting of the sieve-plate unit into the/

the "cap" on its front end (i.e. air-intake end). The front "cap" of the vacuum cleaner was made of aluminium. It was held in position by two lever clasps (shown in Figures 21 and 22), which pressed it firmly on to a rubber-washer mounting so as to give an air-tight joint. Opening of the vacuum cleaner by removal of this "cap" was easy and rapid; the arrangement was retained in the sieve sampler to allow quick changing of culture plates. A central and circular hole 125 mm. in diameter was cut in the aluminium "cap"; the sieve-plate unit was mounted into this hole as shown in Figures 21 and 23. The constructional details of the completed sieve-plate cap are shown in Figure 25, which is a drawing to actual scale of the sieve-plate cap and culture plate in median section and which shows the path of the air being sampled (in red).

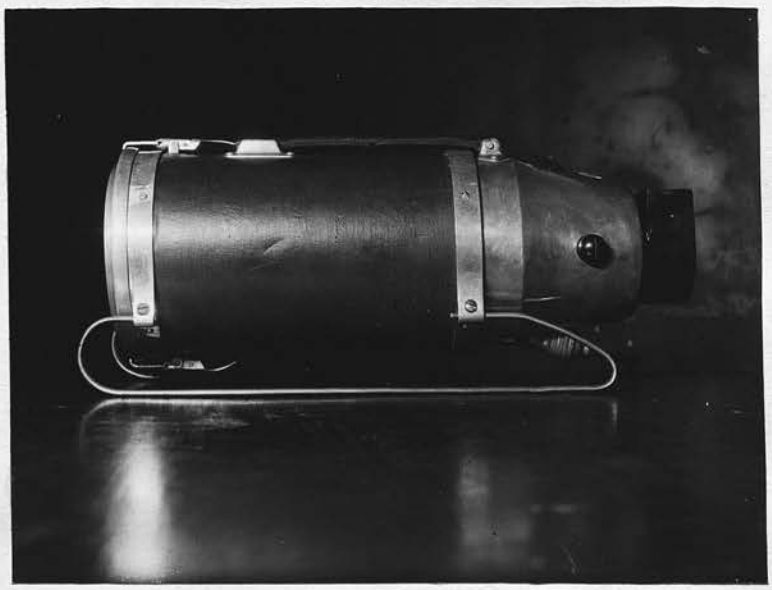
The sieve plate was a circular, flat brass plate, 3 mm. thick and 80 mm. in diameter. It was pierced by 300 vertical holes which were 0.8 mm. in diameter (0.796 mm., drill No.68); these holes were distributed in parallel lines and at uniform spacing over the whole plate. The sieve-plate was mounted at the bottom of a wide and shallow air-intake cylinder; this was 16 mm. deep, 80 mm. in external diameter and 77 mm. in internal diameter. Its free front end was fitted into an 80 mm. diameter hole in a brass front plate which occupied the 125 mm. diameter hole in the aluminium "cap" of the vacuum cleaner.

On/

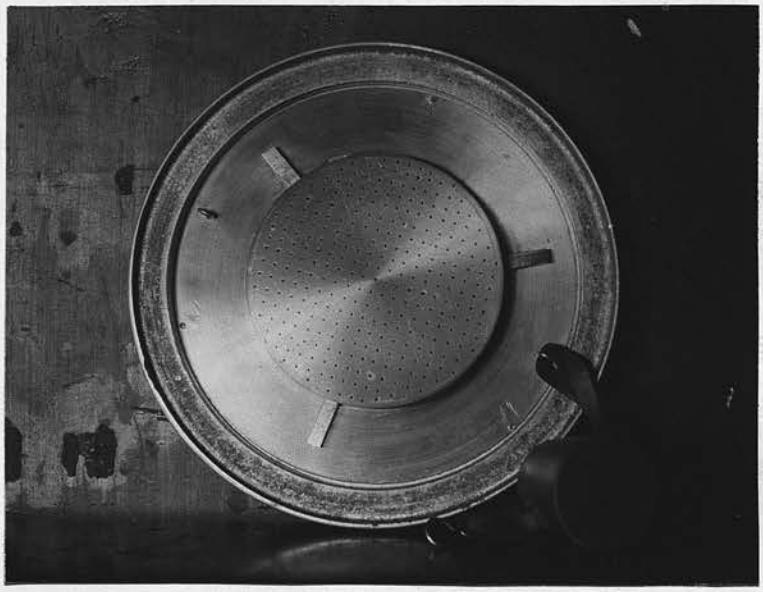
On the periphery of the air-intake cylinder and resting on the back of the front plate, were three brass blocks, or "stops", 15 mm. x 5 mm. x 5 mm.. The free (back) surface of each stop was exactly 8 mm. in front of the free (back) surface of the sieve plate. The function of the stops was to support the rim of the culture plate, as shown in Figures 24 and 25, so that there was a gap of 5 mm. between the rim of the culture plate and the front plate, for free escape of air. The culture plate was filled with agar to the depth of 10 mm. below its rim; constant depth was achieved by use of an indicator in pouring, as shown in Figure 26. Thus, when the culture plate was in position on the stops, the surface of the medium was 2 mm. away from the surface of the sieve plate (usually increased to 2¼ mm. or even 2½ mm. by shrinkage of medium on drying of the plate). The culture plate was held in position by pressure of a rubber stopper beneath a strong rubber band which was attached at its ends to two hooks set at opposite edges of the front plate (see Figure 24). The culture plates had an internal diameter of about 90 mm. (3½ in.), so that when fitted over the sieve plate (80 mm. diam.) they left a gap 5 mm. wide all round the periphery for the escape of air.

Figure 21:- Photograph of Sieve Sampler No.3 Showing the Sieve-Plate Air Intake at the Front End.

Figure 22:- Photograph of Sieve Sampler No.3 Showing the Sieve-Plate Cap Held on by Two Lever Clasps at the Front End (Left) and the Air Exhaust Deflector at the Extreme Rear End (Right).



Figures 23 and 24:- Photographs of Sieve-Plate Cap from Inside Showing the Three Petri Dish Supporters and the Rubber Band with Stopper for Holding the Dish in Place; Upper Photograph Shows Cap without Dish; Lower Photograph Shows Cap with Agar-Containing Dish in Position for Sampling.



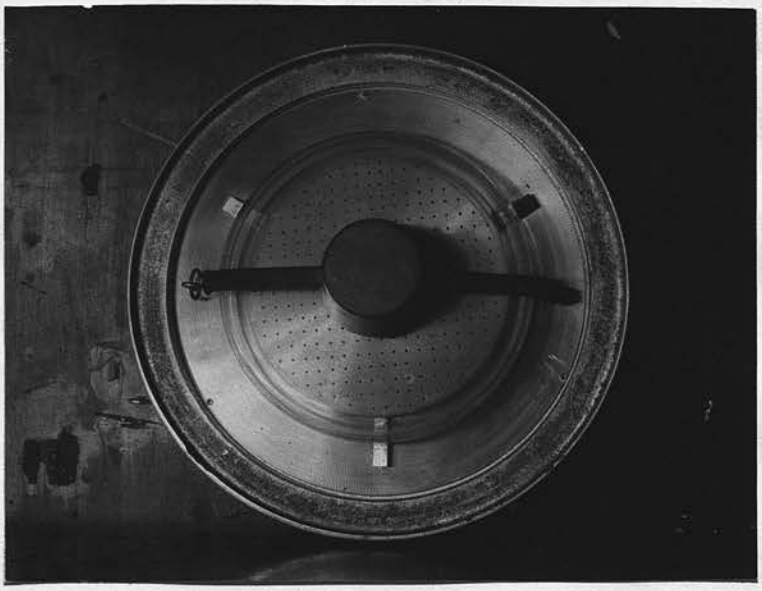


Figure 25:- Diagram to Actual Scale of Median Section of Sieve-Plate Cap and Agar Culture Plate.

(Course of air flow is shown by red lines)

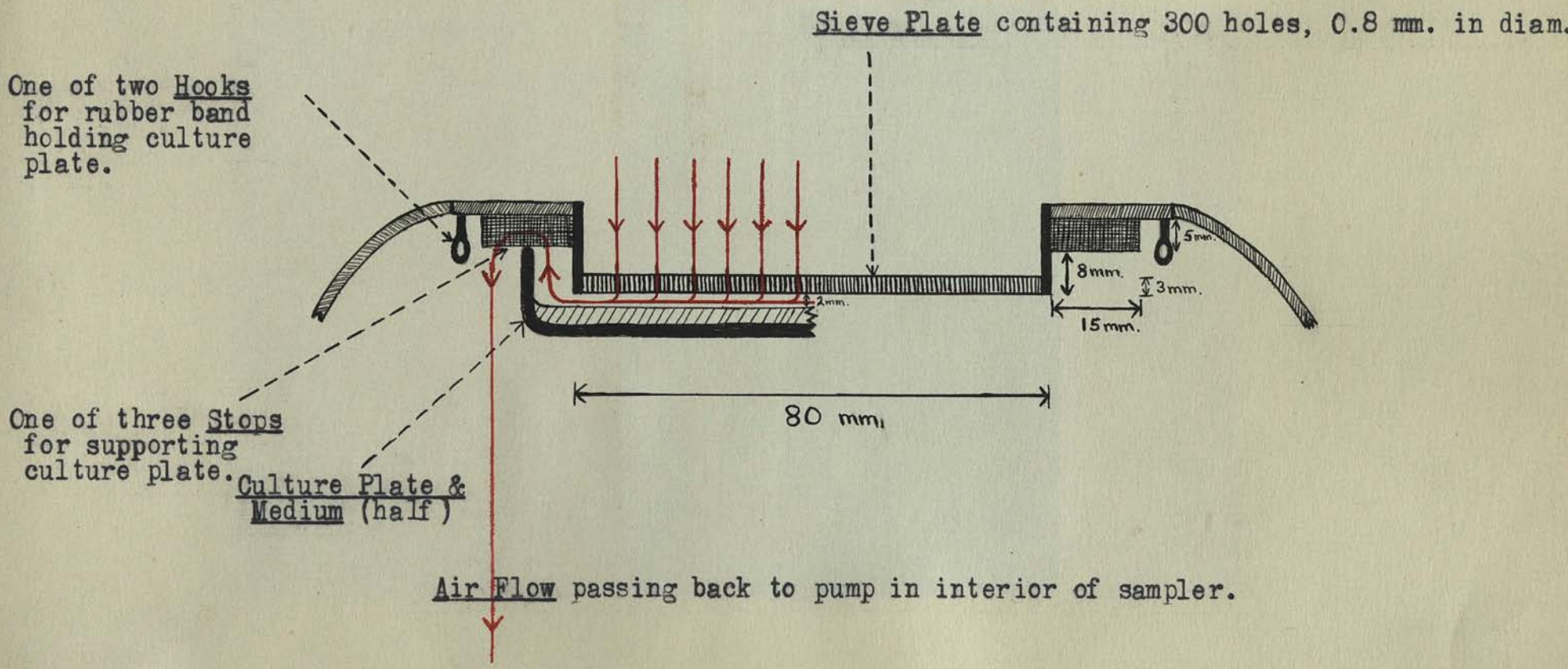


Figure 26:- Photograph Illustrating Method of Pouring Agar Plates to Constant Depth.

Section 3: Calibration of Rate of Air Flow.

The rate of air intake of the sieve sampler was measured by use of a vane anemometer (Negretti and Zambra, London: No.9273). This instrument measures in linear feet the air which passes it. The anemometer was placed against the air-intake orifice of the sieve sampler, as shown in Figure 27; the guard rim of the anemometer was fitted within the sampler orifice to the depth of a few millimeters.

On each occasion the rate of air intake was measured as follows. The sampler was loaded with a culture plate. The electric motor was run for a minute or so before taking the first reading. Then the passage of 100 feet of air into the sampler was observed with the anemometer and the time of this was measured with a stopwatch. Six such readings were taken consecutively for each culture plate tested and the average time for passage of 100 feet was calculated. The volume of air passed in this "average time" was calculated by multiplying 100 feet by the area of cross-section of the sampler's air-intake orifice; from this, the number of cubic feet of air passed per minute was readily calculated. Thus, for sampler No.3, the rate of air intake in cubic feet per minute was given by 304 divided by the average measured time in seconds for passage of 100 feet of air (for samplers No.s 1 and 2, which had slightly smaller intake orifices, read 292 instead of 304).

Many/

Many observations were made, each with a different culture plate poured to give a sieve-agar distance of 2 millimeters. Some observations were made with culture plates poured to give other sieve-agar distances, so as to ascertain the influence of this distance on the rate of air flow. Several observations were made on each day with each sieve sampler, and observations were made on many different days in order to ascertain the full range of variation in the suction capacity of the pump and motor.

The air-intake measurements for a sieve-agar distance of 2 millimeters are shown in Table 91. The overall average rates of sampling were 19.0 cu.ft. per minute for sieve sampler No.1, 23.3 cu.ft. per minute for sieve sampler No.2, and 21.6 cu.ft. per minute for sieve sampler No.3. For the three samplers, the maximum variations in rate of sampling were, respectively, 17.1 to 22.3 cu.ft. per minute, 19.6 to 26.3 cu.ft. per minute, and 20.0 to 22.9 cu.ft. per minute. Very few of the observed air-intake rates were more than 10% above or below the average for the particular sampler; inaccuracy resulting from such a variation was considered unimportant.

The comparative observations made for different sieve-agar distances, are shown in Table 92. The rate of air intake was little reduced by the presence of a culture plate at all sieve-agar distances down to 2 millimeters, but was considerably reduced by a culture plate/

plate at the distance of 1 millimeter. For this reason, routine air sampling was always carried out at a sieve-agar distance of 2 to 2½ millimeters; a small variation in distance at this range was considered unlikely to influence greatly the rate of air intake.

Table 21:- Calibration of Sieve Sampler Air Flow in Cubic Feet per Minute by Vane Anemometer.

(All readings made for sieve-agar distance of 2 mm.)

Sieve Sampler No.1	Sieve Sampler No.2	Sieve Sampler No.3
20.4	24.0	20.0
20.0	24.8	21.0
19.6	19.6	22.9
19.6	25.0	22.5
19.7	24.6	21.1
18.3	23.2	21.3
18.3	23.2	22.2
19.1	24.3	
17.1	24.1	
16.9	24.3	
18.1	24.5	
17.7	24.1	
17.5	24.3	
18.6	23.8	
18.4	24.1	
17.3	24.1	
18.7	24.3	
18.5	23.2	
18.7	25.2	
18.3	25.8	
18.5	26.3	
18.9	25.4	
17.5	20.8	
19.0	24.1	
19.0	21.8	
20.4	20.4	
22.3	21.0	
19.5	21.6	
20.1	21.2	
19.5	20.8	
19.3	22.3	
20.6	20.3	
20.0	23.3	
19.3	21.6	
Average:- 19.0	23.3	21.6

Each reading was made with a different agar plate. The separate sections of the table which are marked off by horizontal lines represent observations made on different days.

Table 92:- Air Flow in Cubic Feet per Minute through Sieve Samplers No.1 and No.3 at Different Sieve-Agar Distances.

	Sieve-Agar Distance in Millimeters				No Agar Plate
	1 mm.	2 mm.	3 mm.	4 mm.	
SIEVE SAMPLER NO.1		20.4 20.0 19.6 19.6 19.7	20.4 20.0 20.3 20.1		20.4
		18.3 18.3 19.1	18.6 18.0 19.1		
	12.4 11.1	17.1 16.9	16.9 17.5	17.4	18.5 18.7 17.8
	14.6	18.1	17.2 17.2	18.1 16.9	17.7 19.6
	14.5 16.2 14.8	17.7 17.5	17.3 17.6	17.4 18.0	17.7 19.0 19.0
Average:-	13.9	18.6	18.4	17.6	18.9
SIEVE SAMPLER NO.3		20.0 21.0	21.5 21.1	21.4	22.0 21.7
		22.9 22.5	22.9	23.2	23.8 22.9
	15.5	21.1	23.0	22.5	23.2
	19.9	21.3 22.2	22.7 22.8	22.8 23.2	22.8 23.6
Average:-	17.7	21.6	22.3	22.6	22.9

Each reading was made with a different agar plate.
The sections of the table marked off by horizontal lines represent observations made on different days.

Figure 27:- Photograph of Vane Anemometer Applied to Sieve-Plate Air Intake for Calibration of Sieve Sampler Air Flow.

Section 4: Calibration of Collection Efficiency.

The collection efficiency, or recovery efficiency, of the sieve samplers was estimated by comparison with a slit sampler. The slit sampler was considered very suitable as a standard for comparison because it has a very high collection efficiency, recovering over 94% of even the smallest bacteria-carrying particles (Bourdillon, Lidwell and Thomas, 1941).

As collection efficiency may vary with the size and kind of infected airborne particles, efficiency measurements were made separately for (i) newly raised dust containing both large and small particles, (ii) small dust particles which remained airborne for 35 minutes after their liberation into the air, and (iii) small droplet nuclei which remained airborne for 90 minutes after their production by sneezing.

The observations were made in the 100-cu.ft. test chamber described in Part 3 (Figure 10). The slit sampler was situated outside the chamber and drew air through the chamber wall from an intake opening at 3 feet above the floor. One or more of the sieve samplers were placed inside the chamber with their air intakes at 3 feet above the floor.

(i) Estimating Collection Efficiency for Large and Small Bacteria-Carrying Dust Particles from Skin and Clothing. The observer wearing his ordinary (non-sterile) clothes entered the chamber and loaded culture plates into the sieve samplers. These movements liberated/

liberated a sufficient amount of infected dust into the air. At once the observer left the chamber, closed the door and put the samplers into operation; the slit sampler and the sieve samplers were run simultaneously during 1 minute. In all cases, the slit-agar and sieve-agar distances were 2 millimeters. Ordinary blood agar plates were used. After incubation at 37 deg.C. for 24 hours, the colonies on each plate were counted with aid of a (x 20) binocular plate microscope.

The results obtained in 19 experiments are shown in Table 93. The colony counts per 1-minute sample are shown in the first three columns. The colony counts per cu.ft. of air were calculated from the 1-minute counts by dividing the latter by the number of cubic feet of air sampled per minute by the sampler in question (average results from Table 91; see Section 3). The count per cu.ft. for each sieve sampler was then calculated as a percentage of the count per cu.ft. for the slit sampler; these percentages are shown in the last two columns of Table 93; they represent the collection efficiencies of the sieve samplers for large and small bacteria-carrying dust particles.

The average collection efficiency of sieve sampler No.1 was 46% and that of sieve sampler No.2 was 47%. The individual collection efficiency values shown in the different experiments ranged from 26% to 81% for sampler No.1, and from 30% to 70% for sampler No.2. This wide variation is perhaps in part due to the smallness/

smallness of the slit sampler counts which makes maximum the effect of random sampling errors.

(ii) Collection Efficiency for Small Bacteria-Carrying Dust Particles from Skin and Clothing.

The observer wearing his ordinary (non-sterile) clothing entered the test chamber and loaded culture plates into the sieve samplers. Then, while still in the chamber, he "marched" during 10 minutes to produce a large amount of dustborne infection of the air. An interval of 35 minutes was allowed between the end of "marching" and the beginning of sampling; in this interval the large dust particles would settle to the floor and when sampling was begun, only the small particles would still be present in the air. After the 35-minute interval, a 5-minute (5 cu.ft.) sample was taken with the slit sampler and a 1-minute sample was taken by each of the sieve samplers during the third minute of operation of the slit sampler (i.e. 19 cu.ft. by sieve sampler No.1, and 23 cu.ft. by sieve sampler No.2). The counts per cu.ft. of air were calculated, and those for the sieve samplers were expressed as percentages of that for the slit sampler; these percentages represented the collection efficiencies of the sieve samplers for small bacteria-carrying dust particles. The results of 5 experiments are shown in Table 94. The average collection efficiency of sieve sampler No.1 was 49%, and that of sieve sampler No.2 was 53%.

(iii) Collection Efficiency for Small Bacteria-Carrying Droplet Nuclei/

Carrying Droplet Nuclei. The observer loaded the sieve samplers with culture plates at some considerable time (e.g. 1-2 hours) prior to the experiment; the chamber air was thus allowed to become free from infected dust. After this interval the observer opened the chamber door and, without entering, delivered four simulated sneezes into the chamber; he then shut the chamber door. The chamber was left undisturbed for a further 90 minutes before air sampling was begun. Only the very smallest bacteria-carrying droplet nuclei could still be airborne after this period of settling (probably only from 1 to 4 microns in diameter). At the end of the 90-minute interval, a 5-minute (5 cu.ft.) sample was taken with the slit sampler and a 1-minute sample was taken with each of the sieve samplers during the third minute of operation of the slit sampler (i.e. 19 cu.ft. by sampler No.1 and 23 cu.ft. by sampler No.2). Blood agar plates were used. After incubation at 37 deg.C. for 24 hours, the colonies on each plate were counted.

The colony counts obtained per sample in 19 experiments are shown in Table 95 (first three columns). The counts per cu.ft. of air were calculated and those for the sieve samplers were expressed as percentages of that for the slit sampler; these percentages represented the collection efficiencies of the sieve samplers for small bacteria-carrying droplet nuclei. The average collection efficiency of sieve sampler/

sampler No.1 was 61% and that of sieve sampler No.2 was 67%. The individual collection efficiency values given in different experiments varied from 33% to 108% for sampler No.1, and from 29% to 103% for sampler No.2.

It is concluded that the sieve samplers operated with about 50% to 60% of the collection efficiency of a slit sampler; as the collection efficiency of a slit sampler approaches 100%, the absolute collection efficiency of the sieve sampler must be about 50%. This 50% collection efficiency of the modified sieve sampler compares favourably with the collection efficiencies of most other samplers, excepting the slit sampler; for instance, the collection efficiency of the widely used "air centrifuge" (Wells, 1933) is only 24% to 41% for small bacteria-containing droplet nuclei (Phelps and Buchbinder, 1941).

Table 93:- Calibration of Recovery Efficiency of Sieve Samplers (S.S.No.1 and S.S.No.2) for Airborne Bacteria-Carrying Dust Particles by Comparison with Recovery by Slit Sampler.

Exp. No.	Colony Counts for Simultaneous One Minute Samples			Efficiency of Sieve Samplers as % of Recovery by Slit Sampler	
	Slit Sampler: 1 cu.ft.	Sieve Sampler 1 19 cu.ft.	Sieve Sampler 2 23 cu.ft.	S.S.No.1	S.S.No.2
1	23	267	-	61%	-
2	37	230	-	33%	-
3	20	155	-	41%	-
4	20	200	-	53%	-
5	30	310	-	54%	-
6	46	711	-	81%	-
7	52	600	837	61%	70%
8	22	125	173	30%	34%
9	21	153	208	38%	43%
10	30	-	303	-	44%
11	21	212	263	53%	54%
12	28	204	264	38%	41%
13	21	-	240	-	49%
14	37	185	253	26%	30%
15	32	258	446	43%	61%
16	34	257	420	40%	54%
17	59	463	646	41%	48%
18	61	433	529	37%	38%
19	41	349	446	45%	47%
		Average:-		46%	47%

The air was infected with dust from the observers skin and clothing by bodily movement immediately prior to taking samples; experiments made in 100 cu.ft.chamber.
Blood agar plates were used.
The sieve-agar distance was 2 millimeters in all cases.
The horizontal lines mark off observations made on different days.

Table 94:- Calibration of Recovery Efficiency of Sieve Samplers (S.S.No.1 and S.S.No.2) for Small Airborne Bacteria-Carrying Dust Particles by Comparison with Recovery by Slit Sampler.

Exp. No.	Colony Count per Sample			Efficiency of Sieve Samplers as % of Recovery by Slit Sampler	
	Slit Sampler 5 cu.ft. in 5 min.	Sieve Sampler 1 19 cu.ft. in 1 min.	Sieve Sampler 2 23 cu.ft. in 1 min.	S.S.No.1	S.S.No.2
1	183	313	438	45%	52%
2	203	416	540	54%	58%
3	352	602	799	45%	50%
4	123	251	277	54%	49%
5	324	587	816	48%	55%
			Average:-	49%	53%

Sieve samplers were run during the third minute of the 5-minute slit sampler run.
Air of 100 cu.ft. chamber was infected with dust from observer's skin and clothing by vigorous body movement ("marching") for 10 minutes.
Larger dust particles were allowed to sediment from the air for 35 minutes before sampling was begun.
The sieve-agar distance was 2 millimeters in all cases.
Heated blood agar plates were used.
The horizontal lines mark off observations made on different days.

Table 95:- Calibration of Recovery Efficiency of Sieve Samplers (S.S.No.1 and S.S.No.2) for Small Airborne Bacteria-Containing Droplet Nuclei by Comparison with Recovery by Slit Sampler.

Exp. No.	Colony Count per Sample			Efficiency of Sieve Samplers as % of Recovery by Slit Sampler	
	Slit Sampler 5 cu.ft. in 5 min.	Sieve Sampler 1 19 cu.ft. in 1 min.	Sieve Sampler 2 23 cu.ft. in 1 min.	S.S.No.1	S.S.No.2
1	116	334	-	76%	-
2	25	-	74	-	64%
3	520	964	1043	49%	44%
4	46	122	149	70%	70%
5	569	964	-	45%	-
6	95	358	452	99%	103%
7	59	124	-	55%	-
8	60	95	146	42%	53%
9	33	100	139	80%	92%
10	156	410	498	69%	69%
11	308	419	970	36%	69%
12	373	458	504	33%	29%
13	112	331	433	78%	84%
14	288	633	944	58%	72%
15	128	258	375	53%	64%
16	40	164	178	108%	97%
17	36	56	86	41%	52%
18	84	196	243	61%	63%
19	88	124	182	37%	45%
			Average:-	61%	67%

The sieve samplers were run during the third minute of the slit sampler run.
The air of the 100 cu.ft. chamber was infected with droplet nuclei by a single simulated sneeze.
The larger droplet nuclei were allowed to sediment from the air for 90 minutes before sampling was begun.
The sieve-agar distance was 2 millimeters in all cases.
Blood agar plates were used.
The horizontal lines mark off observations made on different days.

Section 5: Summary.

A description is given of the design, construction, use and calibration of a new instrument for the bacteriological examination of air. The design of this instrument was based on that of the "sieve-plate sampler" of Du Buy and Crisp (1944). The main modifications on this design procured simpler construction, greater portability and a much greater sampling rate, namely 20 cu.ft. per minute instead of 1 cu.ft. per minute.

Calibration of this "modified sieve sampler", by comparison with a Bourdillon slit sampler, showed that its collection efficiency was about 50% to 60% for large and small bacteria-carrying dust particles and for small bacteria-carrying droplet nuclei.

This "modified sieve sampler" is recommended as a cheap, simply operated, highly portable air sampler of satisfactory collection efficiency and high sampling rate; it seems especially suitable for observation of air infection in hospitals, schools and other places outside the laboratory, and also for laboratory use when a very high rate of sampling is required.

Section 6: References.

Bourdillon,R.B., Lidwell,O.M. and Thomas,J.C. (1941) J.Hyg.,41:198.

Du Buy,H.G. and Crisp,L.R. (1944) U.S.Publ.Hlth.Rept., 59:829.

Phelps,E.B. and Buchbinder,L. (1941) J.Bact.,42:321.

Wells,W.F. (1933) Amer.J.Publ.Hlth.,23:58.

GENERAL SUMMARY

A detailed summary of observations has been given at the end of each part of the experimental record; a general summary indicating the scope of the various investigations, is given below.

PART 2:-

The secretion droplet-spray produced by sneezing, coughing, speaking, laughing, breathing and other expiratory activities was studied by a variety of microscopical and cultural techniques. In particular, a new technique was developed whereby droplet-nuclei could be observed, counted and measured microscopically; droplet-nuclei were thus demonstrated for the first time in photomicrographs.

Measurements were made of the numbers of droplets and droplet-nuclei produced by different kinds of expiratory activity, the proportions of droplets and nuclei which originate from different parts of the respiratory tract, the sizes and size-frequency distributions of the droplets and nuclei, the proportions of droplets and nuclei containing commensal bacteria and the numbers of these bacteria in droplets and nuclei of each size, the proportions of droplets and nuclei likely to contain pathogenic bacteria, the duration of air-carriage and the sedimentation rate of droplets and nuclei of each size, the projection

distance/

distance of droplets and the travelling distance of nuclei, the time required for droplet evaporation and droplet-nucleus formation, and the maximum size of droplet capable of becoming a droplet-nucleus.

It was found that speaking, coughing and sneezing each expels from the front of the mouth many salivary droplets which are small enough to evaporate at once to form minute droplet-nuclei capable of remaining suspended in the air for several minutes or several hours. However, by calculation it was shown that only a small proportion of the droplet-nuclei will contain any pathogenic bacteria, even when an exceptionally large number of these is present in the secretions being atomised.

PART 3:-

Infection of the air by liberation of dust from a person's skin and clothing was studied by making slit-sampler observations in a small test-chamber. Measurements were made of the numbers of bacteria-carrying dust-particles liberated during different kinds of bodily activity, and of the duration of air-carriage of the infected dust-particles. These measurements indicated that dust-particles from the skin and clothing will be comparable to sneeze-produced droplet-nuclei in providing the physical means for airborne transmission of infection.

Salivary streptococci were found on a small proportion/

proportion of the dust-particles, proving that the respiratory-tract bacteria normally are disseminated in this way via the skin and clothing into the air.

It was found that the air contamination with dustborne bacteria from the skin and clothing was reduced only a little by the wearing of a sterile loose surgical gown, but was reduced very much by the wearing of a sterile "dust-proof gown" which was designed specially for this purpose.

PART 4:-

Observations were made of the dissemination of certain pathogenic bacteria by infected patients and "healthy carriers". Examination of a microscope slide or culture plate held at 3 inches in front of the mouth revealed that in the droplet-spray from 6 coughs, M.tuberculosis was expelled by 10 out of 20 patients with open pulmonary tuberculosis, C.diphtheriae by 10 out of 50 patients with faucial diphtheria, Strept.pyogenes by 39 out of 87 persons with scarlet fever or chronic tonsillitis, and Staph.aureus by 1 out of 2 healthy nasal carriers. Only 5 to 10% of the collected cough-droplets contained the specific pathogenic bacteria. Micrometric measurement of the M.tuberculosis-containing cough-droplets from tuberculous patients revealed that 25% of these (each containing 1-10 tubercle bacilli) were small enough to be capable of remaining airborne as droplet-nuclei.

In/

In special experiments with throat carriers of Strept.pyogenes and nose carriers of Staph.aureus, it was found that the air could be infected more readily and to a greater degree by the raising of dust from the skin, clothing, handkerchief, bedding and floor, than by the droplet-spray of speaking, coughing and sneezing.

In the case of two nasal carriers, repeated observations were made, during an 8-week period, of the distribution of Staph.aureus on to the hands, other body surfaces, different articles of clothing and the handkerchief, in the projectile droplets of cough-spray and sneeze-spray, in airborne droplet-nuclei produced by sneezing, and in airborne dust liberated from the skin and clothing.

PART 5:-

By use of a slit sampler and of "settling dishes", bacteriological examinations of the air were made in rooms of a residential training institution during an epidemic of Strept.pyogenes throat infections and in rooms of a hospital maternity-unit having a high incidence of Staph.aureus infections among both the mothers and the babies.

In the training institution a total of 875 cu.ft. of air, in 150 samples, yielded 178 particles bearing Strept.pyogenes, that is 0.2 per cu.ft.. It was calculated that a person living in the institution at the height of the epidemic must have inhaled about 100 infected/

infected particles per day. Strept.pyogenes was found to comprise only 0.5% of all the airborne bacteria. The amount of air infection in a room varied greatly from time to time throughout a day, generally in proportion with the number of persons present and the amount of their movements.

In the maternity unit, "settling dish" examinations revealed the presence of Staph.aureus in the air on 36 out of 40 days during a 4-month period. On 7 of the days, a total of 2125 cu.ft. of air was examined with the slit sampler and was found to contain 85 particles bearing Staph.aureus, that is 0.04 per cu.ft.. It was calculated that an adult inmate of the hospital must have inhaled on average about 12 infected particles per day, and a baby about 1 per day. Staph.aureus was found to comprise only about 0.5% of all the airborne bacteria.

PART 6:-

A modified "sieve sampler" was designed and constructed for rapid bacteriological examination of large volumes of air; the instrument was cheap, simple to operate, highly portable, and sampled at the rate of about 20 cu.ft. per minute. By comparison with a Bourdillon slit sampler, the "sieve sampler" was found to have a collection efficiency of about 50 to 60% for bacteria-carrying particles of the largest and smallest sizes normally found in air.