

**The distribution of micro-organisms in air / by Percy F. Frankland ;
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Distribution of Micro-organisms in
Air.

by

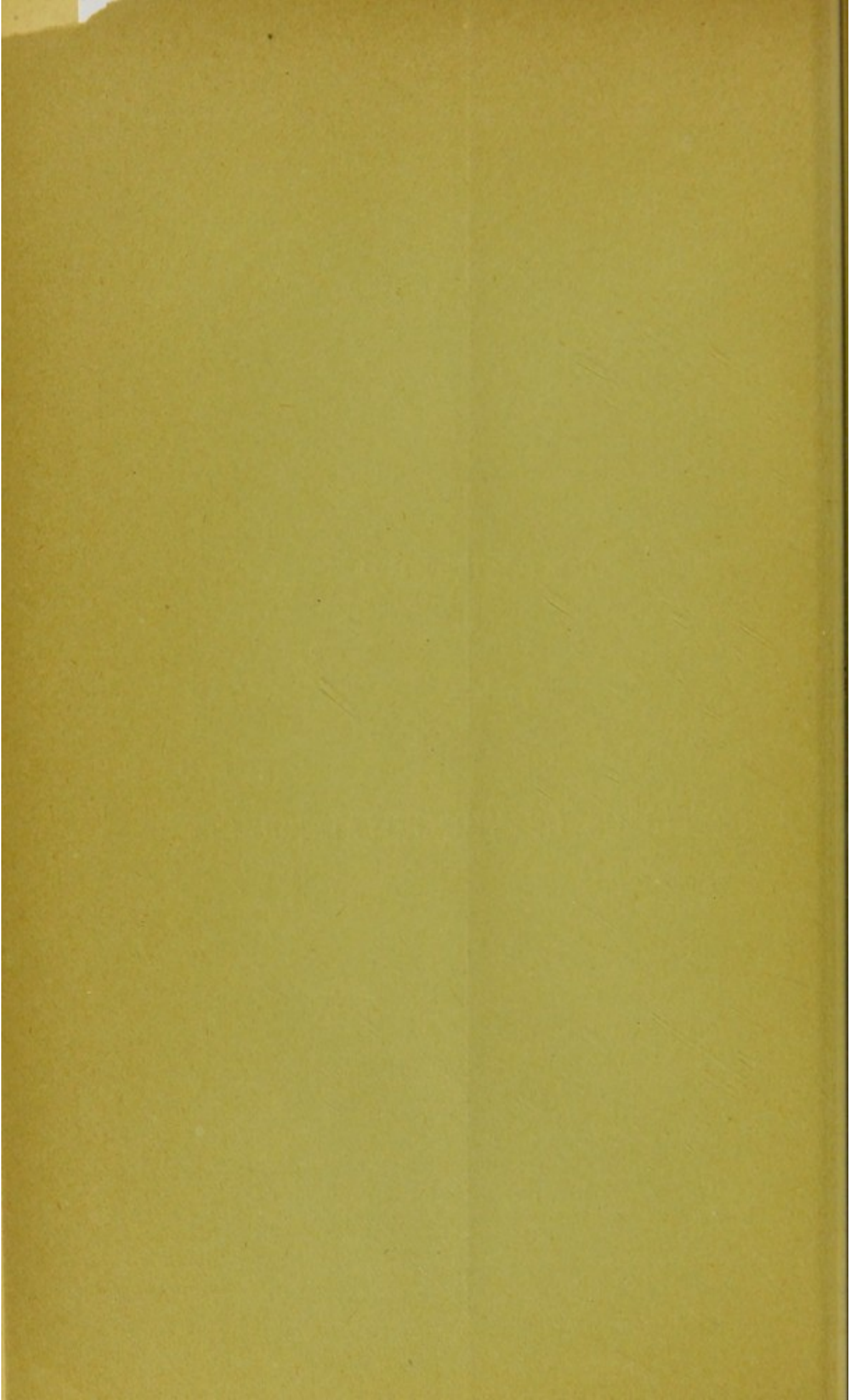
P. F. Frankland.

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"The Distribution of Micro-organisms in Air." By PERCY F. FRANKLAND, Ph.D., B.Sc., F.C.S., F.I.C., Assoc. Roy. Sch. Mines. Communicated by E. FRANKLAND, F.R.S. Received June 7, 1886.

The micro-organisms in air have formed the subject of investigations by Pasteur, Tyndall, Miquel, and many others. The researches of these experimenters have shown that although these organisms are most widely distributed throughout the accessible regions of the atmosphere, yet that very marked differences do exist in the numbers which are present in different places, and in the same places at different times.

The majority of these experiments were made before the methods of cultivating micro-organisms on solid nutritive media had been developed and perfected. These methods of cultivation have proved of such inestimable advantage in the study of micro-organisms in all its branches, that it is not surprising that they should have also been applied to a reinvestigation of the micro-organisms of the air. These methods are, moreover, particularly fitted for this purpose, inasmuch as they simultaneously supply information as to the number of micro-organisms present, as well as furnishing these micro-organisms in a state of pure cultivation for purposes of further study.

A method of adapting the solid culture-media to the bacterioscopic examination of air has been devised by Hesse ("Mittheilungen Kaiserl. Gesundheitsamte," Berlin, 1884), who has conducted a number of experiments showing that the results obtained by this method can lay claim to a fair degree of quantitative accuracy, and since the gelatine-peptone of Koch is used for the cultivation of the organisms obtained, the range of organisms which are capable of being discovered in this way is very considerable indeed.

Wishing to conduct some experiments on the relative abundance of micro-organisms in the air of various places and at different altitudes, I selected, after careful consideration and preliminary trial, the method of Hesse for their execution, and have adopted the various precautions which he has recommended so as to render my results as comparable as possible with those which he has obtained in his investigation on the air of Berlin.

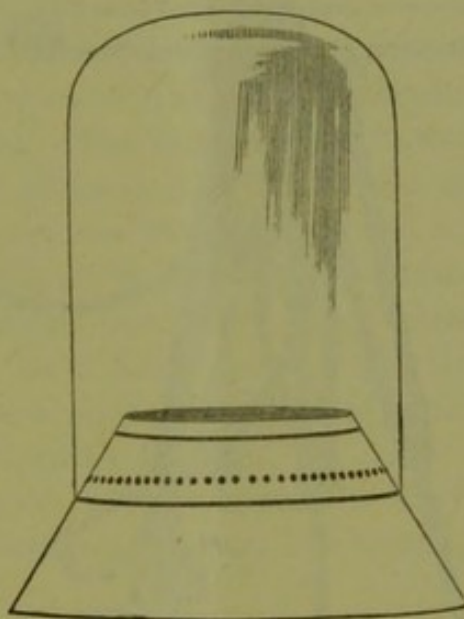
Description of Apparatus employed.

This consists essentially of a glass tube about 2 feet 6 inches in length and $1\frac{1}{2}$ to 2 inches in diameter, coated on its internal surface with the nutritive gelatine medium. One extremity of this tube is fitted with a perforated aperture 0.5 inch in diameter, the other extremity being provided with a tightly fitting india-rubber cork, through which passes a short glass tube plugged with cotton-wool.

In preparing the tube for use, the perforated cap mentioned above is covered with a second non-perforated one, which is tightly wired on so as to be watertight. The empty tube with its caps and cork are first sterilised by placing them in the steamer for several hours, the cotton-wool plug employed in the tube which passes through the cork having been previously sterilised by heating in an air-bath until it is browned. The cork is now removed, and about 50 c.c. of the melted peptone-gelatine are poured into the tube. The cork is replaced, and the whole tube with its contents is then steamed for fifteen minutes on three successive days. In this sterilisation the tube is placed, with the capped end downwards, in the ordinary steamer, the lid of which is replaced by a truncated conical shade,

through which several of these tubes can protrude, their ends being then covered by a glass shade externally coated with cotton-wool or any other non-conducting material. By contriving this simple arrangement I was able to keep every portion of the tubes in an atmosphere of steam, whilst no condensed water could find its way into the tubes.

FIG. 1.



I have found it very necessary not to overdo the steaming, as the melting point of the gelatine is considerably reduced by its being prolonged, and in these experiments it is of the greatest importance that the gelatine should be capable of resisting the temperatures which are incidental to the experiments, and which are encountered in travelling with the apparatus. If the gelatine be too sensitive to heat many experiments may be entirely lost, and the time and labour spent upon their careful execution wasted. I would therefore recommend that both in the preparation and in the sterilisation of the gelatine-peptone used in these tubes, the steaming should be reduced to a minimum consistent with sterility.

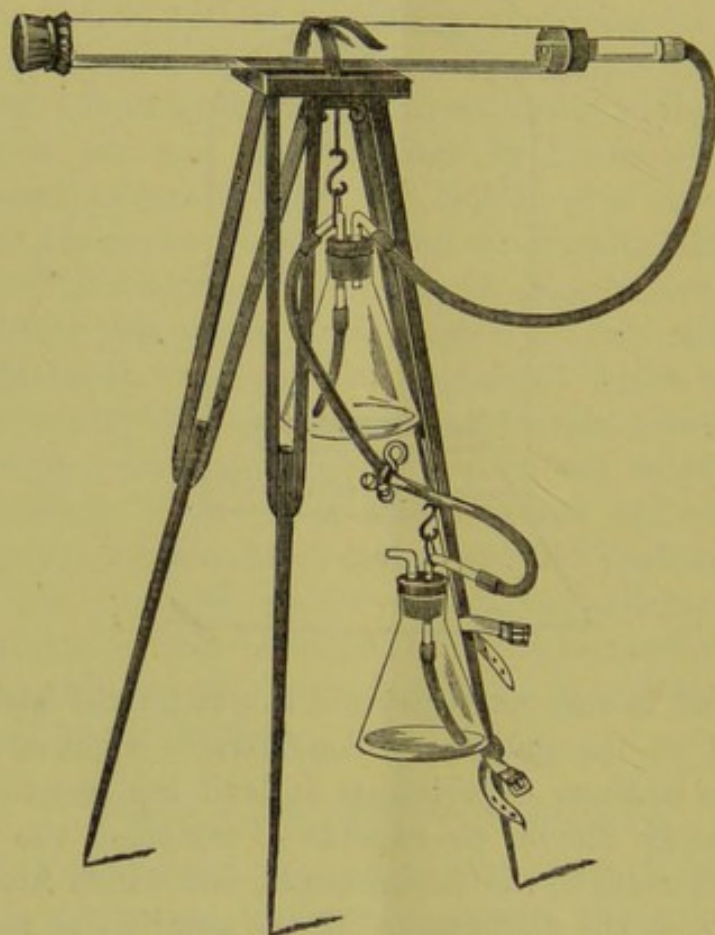
When finally sterilised, and whilst the gelatine is still fluid, the tube is held horizontally under a cold-water tap, being simultaneously rotated and moved backwards and forwards, so that uniform cooling is effected. As the gelatine approaches its solidifying point it becomes more and more viscid, and gradually adheres to the surface of the tube, so that with a little management the whole interior can be uniformly coated. In this operation contact between the gelatine and the cotton-wool plug in the small tube at the end must be carefully avoided, otherwise when cold the tube may become blocked up.

The tube after being preserved for some time to insure sterility, is then fit for use.

Method of Experiment.

Tubes prepared as above are easily transportable in cylindrical cardboard boxes, the exterior of which should be coated white to prevent heating if they have to be exposed to sunshine. The tube is strapped to a small horizontal table supported by an ordinary port-

FIG. 2



able camera stand, and the end of the small tube passing through the cork is connected by flexible tubing with an aspirator, which, so as to render it as portable as possible, consists of two bottles or strong flasks, each of rather more than 1 litre capacity, arranged as in the figure to form a reversible syphon. A measured litre of water is poured into one of these flasks, and by syphoning this into the second, a litre of air is made to pass through the experimental tube. The rate of flow is regulated by a screw clamp, and by alternately connecting the end of the tube with the two syphon flasks any desired volume of air may be drawn through the apparatus.

The experiments of Hesse show that the rate of aspiration should not exceed 1 litre in two or three minutes, and that when this precaution is observed the organisms present in the air are almost wholly deposited in the first two-thirds of the tube, the remaining third being either wholly free or practically so. In my experiments I have almost

uniformly restricted the rate to the 1 litre in three minutes, and can fully confirm the fact of the remarkably complete deposition of the organisms in the front part of the tube, as well as of their being almost uniformly found on the bottom. In conducting experiments in the open air, I have made, with few exceptions, the invariable practice of directing the aperture of the tube at an angle of about 135° to the direction of the wind, so as to avoid currents of air penetrating into the tube irrespectively of the action of the aspirator.

In commencing the experiment the outer unperforated india-rubber cap is removed and carefully folded up, so that its inner surface is not exposed to the air, and as soon as the experiment is over it is carefully replaced and wired on. The tube is kept in a chamber at $20-25^{\circ}$ C. for incubation in the position in which it was used, and in the course of a few days the organisms which have been arrested by the gelatine are readily distinguishable by the colonies, visible to the naked eye or by means of a low power, to which they give rise. Unless the number of organisms which have fallen on the gelatine is very great, each colony will consist of a pure cultivation of a particular organism, and can be further examined as desired.

From the number of colonies found in a given volume of air the number in any standard volume, say 100 litres, can be calculated.

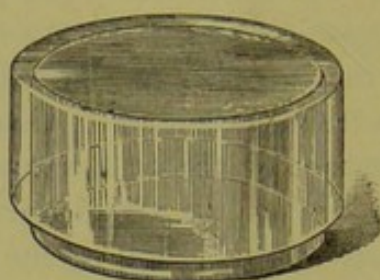
Examination of Air by Gelatine-surface Exposure.

In addition to the tube experiments above described, I have considered it advisable to make simultaneous tests by exposing for a definite period of time a surface of nutrient gelatine of definite area as originally recommended by Koch (*"Mittheilungen Kaiserl. Gesundheitsamte,"* Berlin, 1881), as in this manner an idea is obtained, not of the number of organisms contained in a given *volume* of air, but of what for many purposes is more important, of the number falling on a given surface in a definite time.

For this purpose small circular glass dishes rather less than 1 inch in height and about 3 inches in diameter, and provided with a glass cover fitting loosely and overlapping like the lid of a pill-box, were filled to a depth of about one-third of an inch with nutrient gelatine and sterilised for fifteen minutes on three successive days in the steamer. As long as the covers are on, the gelatine in these dishes remains sterile for practically an indefinite length of time, and can be transported without danger in a tin box.

In using these for experiment, the lid is removed and placed with its mouth downwards on a clean surface, and then after the desired exposure replaced on the dish.

FIG. 3.

*General Scope of the Experiments.*

A number of experiments have been made on the roof of the Science Schools, South Kensington Museum, with the view of ascertaining the influence of season and atmospheric conditions generally upon the abundance of micro-organisms. This position is well fitted for observations of the kind, the roof being about 50 or 60 feet above the surface of the ground, and thus removed from local and accidental influences.

A series of experiments was carried out with a view of ascertaining the relative abundance of micro-organisms at different altitudes in towns. These comparisons were effected by collecting samples of air at different elevations on the spire of Norwich Cathedral, on the dome of St. Paul's in London, and on Primrose Hill. Comparative experiments have also been made in the country, as well as in buildings such as museums, hospitals, &c.

The results obtained in the tube experiments are calculated to the number of micro-organisms contained in 10 litres of air, whilst the results yielded by the exposure of the gelatine-dishes are stated so as to represent the number of micro-organisms falling on 1 square foot in one minute. The conditions under which the experiments were performed are also recorded.

Table I.—Roof of Science Schools, South Kensington Museum.

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
From N.E. window of room on top floor but one. 21st January, 1886.	Wind N.N.E., moderate. Snow on ground and falling slightly during first part of experiment. Temp. 3.5° C. (noon).	10 in 25 litres	4	—
Exhibition Road, at bottom of Science Schools. 28th January, 1886.	Thick white fog lifting. Wind S.W. by W, very moderate. Road wet, pavement drying. The dishes were placed on balustrade 5 ft. above pavement. Temp. 6° C. 11 A.M.	30
Ditto	Ditto.	32
Roof of Science Schools. 28th Jan., 1886.	Conditions identical. Dishes exposed on western (windward) parapet. Temp. 5.5° C. 11 A.M.	26
Ditto	Ditto.	33
Roof of Science Schools. 9th March, 1886.	Cold S.E. wind, strong, had continued for several days previously; everything dry, and roads unwatered; sun shining. Temp. (shade) 3.5° to 5° C. Dishes exposed on E (windward) parapet in full sweep of wind. 3 to 4.30 P.M.	433
Ditto	Ditto.	414
Ditto	The tube was not pointed quite sufficiently away from the wind; the result is therefore, probably, somewhat too high.	135 in 12 litres.	113	—

Table I.—*continued.*

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Roof of Science Schools, 16th March, 1886.	Wind N.W., moderate, ground dry and frosty. Tube pointed S.W. 11.50 A.M. to 12.30 P.M. Temp. 3° C.	13 in 10 litres	13	—
Ditto	Four dishes were simultaneously exposed on W. (windward) parapet.	101 109 83 101
Roof of Science Schools, 31st March, 1886.	Wind S.W. by W., intermittent and variable. Sun shining greater part of time; ground wet. Wind increased consider- ably at 1.45 P.M. Temp. 12.5° to 11° C.	851 215 803 1,302
Ditto	42 in 15 litres	28	—
Ditto	Heavy shower since previous experi- ment. Wind S.W., strong, falling later. Temp. 8° to 9° C.	516 429 375 372
Ditto	Ditto.	61 in 16 litres	38	—
Roof of Science Schools, 12th May, 1886.	Continuous rain previous day until morning of experiment; ground and surroundings thoroughly wet. Wind E., fairly strong. Tube pointed N.W. 4.12 P.M. Temp. 12° C.	66 60 61

Table I.—*continued.*

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Roof of Science Schools, 12th May, 1886.	Continuous rain previous day until morning of experiment; ground and surroundings thoroughly wet. Wind E., fairly strong. Tube pointed N.W. 4.12 p.m. Temp. 12° C.	29 in 12 litres	24	—
Roof of Science Schools, 13th May, 1886.	Intermittent drizzling rain during morning; very heavy rain previous night. Wind S.W., not quite so strong as on previous day. 5.25 to 6.30 p.m. Temp. 12° C.	37 in 12 litres	31	—
Ditto	Ditto.	88
Roof of Science Schools, 22nd May, 1886.	After thunderstorm (3 to 4 a.m.) and abundant rain. Wind fairly strong and irregular in direction, N.E. to S.E. 10.45 a.m. Temp. 16.5° C.	48 in 12 litres	40	—
Ditto	Ditto.	71
Roof of Science Schools, 25th May, 1886.	Wind S.W., moderate. Pavement dry, but heavy rain all previous day and part of night. 6.10 p.m. Temp. 13° C.	32 in 12 litres	27	—
Ditto	Ditto.	130

These figures show that in cold weather, especially when the ground is covered with snow, the number of organisms in the air is very much reduced and presents a very striking contrast to the number found in the warmer weather, even immediately after much rain.

The experiments made on the 9th of March show that during cold and dry weather with a strong east wind blowing over London, a large number of micro-organisms may still be present in the air; for although the tube-experiment made on that day cannot be regarded as satisfactory, yet from the number falling on the square foot it is evident that they must have been very abundant, certainly very much more abundant than on the 16th of March, when with much the same temperature the wind was more moderate and blowing from the north-west.

It is particularly noticeable that even after such exceedingly heavy rain as was experienced on the morning of the 22nd of May, within a few hours afterwards the number of micro-organisms in the air should be as abundant as usual.

It will be seen on comparing the number in the above table that, with the exception of the experiment made on January 21st, when snow was on the ground and actually falling at the time, the number of micro-organisms present in the air collected on the roof of the Science Schools, never fell so low as in some of the experiments to be presently mentioned, which were made in the country.

Taking the average of the experiments recorded above, it will be seen that the mean number of organisms found in 10 litres of air amounted to 35, whilst an average of 279 fell on 1 square foot in one minute.

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Edge of Reigate Hill, about 700 ft. 7th February, 1886.	Wind E., in gusts, but not very strong; ground partially hard with frost and partially moist; occasional sunshine. Noon. Temp. 1° C.	2 in 10 litres	2	15
Garden on Reigate Hill, 200 ft. lower. 7th February, 1886.	Similar, but less wind. Dish exposed on grass lawn. 1.30 P.M. Temp. 5° C.	30
Lawn in garden on Reigate Hill. 23rd May, 1886.	Wind S.W., very gentle. 6 to 7 P.M. Temp. 16° C.	30 in 12 litres	25	31
Edge of Chalk Down, Reigate, about 700 ft. high. 23rd May, 1886.	Mist; wind S.E., strong to moderate; ground wet (grass); air very moist. Dish exposed 3 ft. from ground. Temp. 13° C. Noon.	16 in 12 litres	13	48
Mousehold Heath, Norwich. 23rd April, 1886.	Wind E., moderate, blowing across country. Heath covered with dry heather. Noon; sunshine. Temp. 13° C. Dish placed on stand 3 ft. 6 in. above ground.	7 in 10 litres	7	18
Garden, near Norwich. 23rd April, 1886.	Wind E., blowing across Norwich, less strong than in morning; sunshine. Temp. 12° C. 4 to 5 P.M. Grass.	252
Garden, near Norwich. 25th April, 1886.	Wind N.E., slight. Temp. 4° C. 6 P.M.	100
Mousehold Heath, Norwich. 27th April, 1886.	Wind E.; conditions much as in previous experiment. 11 to 12 A.M.; sunshine.	5 in 10 litres	5	16
Garden, near Norwich. 28th April, 1886.	Wind E., slight; sunshine. Temp. 12° C. Noon.	31 in 10 litres	31	386

In the table (p. 519), the results of the experiments made near Reigate and in the vicinity of Norwich are recorded.

These figures present a very marked contrast to those contained in the previous table; thus the average number of organisms found in the country experiments amounts to only 14 in 10 litres, whilst an average of 79 fell on 1 square foot in one minute.

Particularly noticeable is the great relative freedom from micro-organisms of the air collected on the heath near Norwich during the comparatively warm weather of April last when the ground was dry. In the experiments made both at Norwich and Reigate it will be observed that the air in gardens was richer in micro-organisms than that of the open country, although in the Reigate experiments of May 23rd there were more on the dish exposed on the hill than in the garden, but this is easily accounted for by the fact that the wind on the hill was very much stronger than in the garden, the number found in a given volume being very much less in the case of the air on the hill.

In the table (p. 521) are the results of the examination of the air in Kensington Gardens, Hyde Park, and Primrose Hill.

From these figures it will be seen that on the whole the number of organisms found in the air of these open spaces is less than in that collected on the roof at South Kensington, but greater than in the experiments made in the country, although the number found in the second experiment in Kensington Gardens is scarcely in excess of anything found in the country places.

The average number of micro-organisms found in 10 litres amounts to 24, whilst an average of 85 fell on 1 square foot in one minute.

Table III.—Experiments in Open Places in London.

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Kensington Gardens, near Round Pond. 1st April, 1886.	East side of Round Pond. Wind S.W. by W., fairly strong, blowing across grass; grass and ground damp; streets dry; sunshine. 12 to 2 P.M. Temp. 12° C.	32 in 15 litres	21	88
Ditto	Conditions similar, but wind much less strong. 2.15 to 3.30 P.M. Temp. 11° C.	7 in 15 litres	5	74
Hyde Park. 18th May, 1886.	Grass in hollow. Wind S.W., fairly strong. 5 P.M. Temp. 16° C. to 14.5° C.	57 in 12 litres	48	207
Ditto	Similar. Wind considerably less. 6 P.M. Temp. 14° C.	45 in 12 litres	38	74
Primrose Hill, top. 19th May, 1886.	Ground very wet; grass. Wind S.E., fairly strong. 2 P.M. Temp. 13.5° C.	11 in 12 litres	9	12
Primrose Hill, bottom. 19th May, 1886.	Similar. Wind not so strong as in above. 3 P.M. Temp. 13.5° C.	29 in 12 litres	24	57

Table IV.—Experiments at Different Altitudes.

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Cathedral Spire, Norwich. 26th April, 1886.	Height about 300 ft. Wind E., fairly strong. Tube projecting from S.E. window at top of spire. 5 P.M.	7 in 10 litres.	7	49
Cathedral Tower, Norwich. 26th April, 1886.	Wind E., fairly strong. Tube projecting from northern battlements; sunshine. Temp. 8° C. 4 P.M. Height, 180 ft.	9 in 10 litres.	9	107
Cathedral Close, Norwich. 26th April, 1886.	Wind E., moderate. Gravel space in front of south transept of Cathedral. Ground dry; sunshine. Temp. 9° C. 12 to 1 P.M.	18 in 10 litres.	18	354
Golden Gallery, St. Paul's. 26th May, 1886.	Wind S.W., strong; streets dry. Day fine, with exception of few drops of rain during experiment. 2 P.M. Temp. 12° C.	13 in 12 litres.	11	115
Stone Gallery, St. Paul's. 26th May, 1886.	Wind as above; sunshine. 3.20 P.M. Temp. 13° C.	41 in 12 litres.	34	125
St. Paul's Churchyard, 26th May, 1886.	Pavement in gardens at foot of St. Paul's. 5 P.M. Temp. 12.5° C.	84 in 12 litres.	70	188
Golden Gallery, St. Paul's. 29th May, 1886.	Wind N.W., moderate; hazy. Slight rain during morning. Pavement and road wet at beginning of experiment; slight sunshine. 12.30 P.M. Wind changed completely in direction during experiment to S.W.; blew down aperture of tube. Temp. rose from 11° to 12.5° C.	58 per 10 litres.	58	113
Stone Gallery, St. Paul's. 29th May, 1886.	Wind S.W., strong. 2 P.M. Temp. 13° C.	23 in 10 litres.	23	226

At the suggestion of Mr. Edwin Chadwick, C.B., I was induced to make a comparison between the air at different altitudes in towns. The results obtained are recorded in the above table.

These figures are particularly instructive, as illustrating the fact that with increasing altitude the air becomes poorer in micro-organisms. The tube experiment on the Golden Gallery at St. Paul's on the 29th May must be disregarded, as during the progress of the experiment the direction of the wind changed completely and vitiated the result by blowing down the open extremity of the tube, thereby increasing the number of organisms deposited beyond what was actually due to the volume of air aspirated. The exposed dish, on the other hand, would not be affected in the same way, and it will be seen that, as regards the number of organisms falling on the square foot, the result on the Golden Gallery carries on the diminution noticed on the Stone Gallery.

The differences noticed in the case of the St. Paul's experiments may perhaps be most vividly appreciated by comparing the averages of the two experiments with some of the averages already quoted for other places. Thus at the base of St. Paul's we find an average of 56 organisms in 10 litres, which is considerably greater than the average for the South Kensington experiments, in which only 35 were found in the same volume; on the Stone Gallery there were 29 in 10 litres, whilst in the Golden Gallery in the successful experiment the number only amounted to 11 in 10 litres, thus closely resembling the average number found in the experiments made in country places, which was 14 in that volume.

Table V.—Indoor Experiments.

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Drawing room before children's dance. 6th February, 1886.	Room almost empty. Dishes exposed 4 ft. above the ground.	44 50
Ditto during dancing....	About 20 children dancing, otherwise conditions as above.	400 333
Railway carriage. 28th April, 1886.	Four passengers; one window quite open, one closed. Dish exposed by open window 3 ft. above ground.	395
Ditto	Ten passengers; one window open 4 inches; one window closed. Dish exposed by closed window.	3,120
Norwich Union Life Office. 24th April, 1886.	Room 45 ft. × 27 ft. × 15 ft. high. No windows open, but ventilated by lateral tube. About 10 clerks walking about. Dish exposed about 4 ft. above ground.	78
S. Kensington Museum. Central Hall. Morning. 14th May, 1886 (Friday).	Italian Court, centre. Very few visitors. Temp. 17° C. Dish exposed 3 ft. 6 in. above ground.	20 in 11 litres.	18	20
S. Kensington Museum. Morning. 15th May, 1886 (Saturday).	Same place. Considerably more visitors. Noon. Temp. 17° C.	97 in 12 litres.	81	76
S. Kensington Museum. Afternoon. 15th May.	Ditto, 4 to 5 p.m. Temp. 17·5° C.	78 in 12 litres.	65	97

Place.	Conditions of experiment.	No. of colonies found.	Total number found in 10 litres (calculated).	Total number falling per sq. ft. per minute (calculated).
Natural History Museum. 17th May, 1886. (Monday morning.)	Centre of entrance hall. Noon. Temp. 16.5° C. Dish exposed on pillar at foot of main staircase. Considerable number of visitors passing. Entrance door closed.	Tube lost.	30
Natural History Museum. 17th May, 1886. Afternoon.	Ditto. 3.40 p.m. Temp. 17° C. More visitors than in morning.	Tube lost.	293
Natural History Museum. 21st May, 1886. Morning.	Same place. One entrance door open, causing perceptible draught of air. Number of visitors few. Noon. Temp. 18.5° C.	60 in 12 litres.	50	136
Natural History Museum. 21st May, 1886. Afternoon.	Ditto. Entrance door less open. Number of visitors much greater. 2.25 p.m. Temp. 18.5° C.	84 in 12 litres.	70	255
Hospital for Consumption Brompton. 27th May, 1886.	"Richmond" ward; 8 beds; 8 persons in room. 1 p.m. Temp. 15° C. Windows closed. Lateral ventilation, and door open on to corridor. Dish exposed on chair placed on bed.	43 in 10 litres.	43	11
Ditto	Ditto. 8 persons in room; some moving about. 4.20 p.m. Temp. 16° C. Dish similarly exposed.	130 in 10 litres.	130	130
Ditto	Ditto. 8 persons in bed, 9 p.m. Temp. 16.5° C. Dish exposed on chair on ground.	42 in 10 litres.	42	44
Chemical Laboratory, Science Schools, South Kensington Museum. 13th January, 1886.	Windows shut, door open; 2 persons in room. Noon. Temp. 17° C. Wind and snow outside.	26 in 20 litres.	13	—

These experiments show that in enclosed spaces, when there is little or no aërial commotion, the number of suspended organisms is very moderate, but as soon as any atmospheric disturbance is occasioned either by draughts or by the moving about of people, the number rapidly rises, and may become very large indeed. This is a fact very familiar to all who have had much experience in the cultivation of micro-organisms, and it is of particular importance in connexion with the process of plate cultivation with gelatine.

The experiments made in the railway carriage afford a very striking example of the enormous number of micro-organisms which become suspended in the air when a large number of persons are crowded together.

In my own laboratory, where every care is taken to prevent the circulation of dust, the number of organisms did not amount to more than 13 in 10 litres, and of the comparative purity of this air I have had the most abundant evidence in the almost inappreciable amount of aërial contamination which is exhibited by the plate cultivations prepared there.

In conclusion, I have to express my thanks to the Deans of St. Paul's and Norwich, General Festing, Professor Flower, Dr. Theodore Williams, and Mr. F. E. Colenso, through whose courtesy I have been enabled to carry out many of the experiments recorded in the above paper.

I must also acknowledge the valuable help which I have received from my assistant, Mr. Hart, A.R.S.M., who is now proceeding with these investigations, the results of which we shall hope to have the honour of communicating later.