THE MICROBIOLOGY OF THE UPPER AIR. II[1]

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The more common use of aircraft during recent years has enabled various investigators to make collections of microorganisms occurring in the air at various altitudes in widely separated parts of the world. Such studies have been reported in the United States by Stakman, Henry, Curran and Christopher (1923), Brown (1930), and Meier, Stevenson and Charles (1933). In Canada, Craigie and Popp (1928) have collected rust spores from the air and in Russia some microbiological studies in the upper air were made by Mischustin (1926). More recently Meier and Lindbergh (1935) have published the results of collections made by Colonel Lindbergh in extended flights above the Arctic Circle.

During the past two years collections of this nature have been made in the vicinity of Boston. This has been possible due to the fact that almost daily airplane flights to relatively high altitudes were being conducted under the direction of the Division of Meteorology, Department of Aeronautical Engineering, of the Massachusetts Institute of Technology. These flights were designed primarily for the purpose of obtaining meteorological data at high altitudes, but through the courtesy of the Division of Meteorology, the same flights have sometimes been used for making collections of air micro-organisms.

A description of the apparatus and the results of collections made in 45 earlier flights of this nature have been reported previously (Proctor, 1934). The present report deals with the

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collections made on 30 additional flights carried out between April and November, 1934.

In brief, the collection apparatus was a brass plate capable of being rotated, with six holes drilled through it at equal intervals. In these holes were set disks of lens paper on a wire screen support. The disks were saturated with a hydrocarbon oil with a low congealing point (−71°C.) and acted as the actual trapping or filtering agents. The plate was enclosed in a tight metal drum with an inlet and outlet tube set on the opposite faces so that a current of air drawn through the outlet could pass through each of the six holes, one at a time, by rotating the plate.

The entire apparatus was sterilized by heat before use. During transportation between the laboratory in Cambridge and the flying field of East Boston it was placed in a special sterilized container.

When installed in the plane, the inlet and outlet tubes of the collector were connected to other tubes installed permanently in the plane so that a current of air could be drawn from outside the cabin through the collector and out through a Venturi tube on the roof, which furnished the vacuum necessary for moving the air. The collections could be made by merely turning a knob, thus enabling the sole occupant of the plane to control the collections, in addition to his numerous other duties as pilot and meteorological observer. Additional holes drilled in the rotating plate between the collection disks allowed the unrestricted passage of air through the apparatus when collections were not being made, thus furnishing what might be termed an air-wash of the apparatus between collections or exposures at different altitudes. One of the six collection disks was ordinarily not exposed and therefore acted as a control.

This apparatus does not collect all the micro-organisms from the air passing through it, but offers a means of comparison for collections at various altitudes. A perfect trap would so restrict the air flow as to make its use unsuitable in altitude flights of the nature of those mentioned. Tests made in the M.I.T. wind-tunnel indicated an air-flow through the device of approximately 1 cubic foot per minute under general flight conditions.

Some of the flights were made with a Curtis-Robin plane. The later ones were made with a Fairchild plane which has been assigned for meteorological work at the M.I.T. by the Aviation Corps of the U. S. Army. The flights were made between 7 and 9 a.m.

The laboratory work was usually carried out within a few hours after completion of flight. The oiled paper filters were removed to a specially sterilized glass cell, using aseptic technique. A sterile cover slip was placed over the disks and they were examined directly with a microscope, using a 16 mm. objective and a 10× ocular. By means of a mechanical stage, counting of the visible dust particles and any pollen grains present on the disks was simplified.

After the direct examination, the disks from each altitude were placed in separate tubes containing 10 cc. sterile water and plugged with sterile rubber stoppers, using aseptic precautions. After standing five minutes the tubes were shaken thoroughly until the paper disks were disintegrated into separate fibers. The water from each tube was divided among three or four Petri plates, being transferred by means of sterile pipettes. Standard nutrient agar was added in the usual manner, allowed to cool, and the plates incubated for five days at room temperature. At the end of this period they were placed in a 37°C. incubator for two additional days, after which counts were made.

This technique made possible determination of those organisms capable of growing aerobically on standard nutrient agar at room temperature, also those growing at 37°C.

Tables 1 to 6 contain the results obtained from 30 flights. In addition to the bacteria and mold plate counts, the results of the direct dust counts are included in the last 27 flights. Due to variation in conditions of flight, it was not possible to have all exposures of an equal time interval nor could the altitude brackets be identical.

Such other atmospheric factors as seemed pertinent have been included in the tables. It will be noted that in the temperature column the difference between G (ground temperature) and T (temperature at the top of flight) is often considerable. The

TABLE 1

Flight data

NUMBER	AIR MASSES	WIND, WEATHER	RAINFALL	RELATIVE HUMIDITY	TEMPERATURE	TIME	ALTITUDE (1000 FT.)	BACTERIA	MOLDS	DUST COUNTS
				per cent	°*C.*	*minutes*				
1. April 30, Mon.	N Pc	SSW to W	0	55	G 6.5	10	5–10	4	1	
					T −37.1	11	11–15.5	9	0	
						10	16–20	5	0	
						22	20–25.4–16	15	1	
		Sm				5	15–3	4	1	
Control						0		0	0	
2. May 2, Wed.	N Pc from ocean	S	0	55	G 12.5	10	5–10.5	18	2	
		Cl, Sm			T −25.5	10	11–15.8	30	0	
						10	16–20	12	1	
						10	20.2–22.3	6	0	
						8	19–10	2	1	
Control						0		0	0	
3. May 8, Tues.	Pc	NW	0	57	G 10	10	5–10	1	2	
					T −22.8	10	11–15	1	2	
		SH				10	15.5–21	1	0	
		Waltham				8	0.6–1	7	57	
		Waltham				8	1–1	3	60	
Control						0		0	0	
4. May 11, Fri.	N Ta	W	0.04	87	G 25.3	8	5–10	32	10	641
					T −17.9	9	11–25.5	4	4	225
		Dust storm,				4	16–18	6	2	279
		strong up-				10	17–2.8	68	3	927
		ward cur-				10	2.7–1.5	74	8	562
		rents								
Control						0		0	0	110
5. May 12, Sat.	Pc	NNW	0	53	G 9.9	10	5–10	0	0	271
					T −33.6	10	10–15	1	0	235
		C				12	18–23–16	1	1	186
Control						0		0	0	58

Symbols used in tables 1 to 6 concerning weather refer to observations made at the time of the flight and are as follows: C, clear; Cl, cloudy, O, over-cast; F, fog; R, rainy; Sm, smoky; SH, slight haze; PCl, partly cloudy.

Some of these terms are difficult of strict definition but give an indication of general weather conditions.

The air masses are the results of the analysis of air mass properties determined from the meteorological data obtained on each flight in accordance with the classification of Willett (1933). The following symbols and their significance are explained briefly below.

Pc (Polar Canadian), from the northern continental area. Pp (Polar Pacific), from the north Pacific area. Pa (Polar Atlantic), from the Sargasso Sea area. Ta (Tropical Atlantic), from the Sargasso Sea area. Tg (Tropical Gulf), from the Gulf of Mexico and Caribbean Sea area. N, modified air masses originally of P or T character but of transitioned type.

The bacteria, molds and dust counts as given are the total numbers enumerated from each exposure.

rainfall cited is that precipitation which occurred during the twelve hours preceding each flight. The relative humidity is that determined at ground level at the approximate time of each

TABLE 2*

Flight data

NUMBER	AIR MASSES	WIND, WEATHER	RAINFALL	RELATIVE HUMIDITY	TEMPERATURE	TIME	ALTITUDE (1000 FT.)	BACTERIA	MOLDS	DUST COUNTS
				per cent	°*C.*	*minutes*				
6. May 13, Sun.	N Pc becoming Pa	NNW	0	42	G 11.4	10	5.5–10.5	2	2	338
					T −27.8	7	13–16	1	0	270
						9	16.8–20	5	0	175
		C				20	20.5–23–20.5	2	0	105
Control						0		0	0	35
7. May 14, Mon.	Pp	W	0.02	78	G 14.2	9	5–10	1	0	487
					T −21.8	10	11–15.5	3	1	430
						12	16–20	3	3	310
		O				7	20.3–21.5–20	5	0	195
						8	18.4–10	1	1	225
Control						0		0	0	30
8. May 18, Fri.	N Pc	W	0.01	55	G 13.5	9	5–10	35	5	921
					T −24.7	10	11–15.5	3	0	572
						15	16–20	3	0	383
		P Cl				13	20–22–20	4	0	291
						8	19–10	3	1	178
Control						0		0	0	85
9. May 19, Sat.	Pc	N	0.02	44	G 13.7	8	5–10	7	0	338
					T −24.7	9	10.5–15.5	3	0	287
		C				9	16.0–21.0	0	1	215
Control						0		0	0	75
10. May 21, Mon.	N Pa	N	0	88	G 15.9	8	5–10	0	1	521
					T −17.3	12	11–15.5	0	0	1225
		O				15	16–20	1	0	475
						9	19–20–10	0	1	438
Control						0		0	0	40

* See footnote to table 1 for explanation of symbols.

flight. The wind is also that occurring at the lower levels on the morning of the flight but may not be the same at all levels.

The data included in these tables suggest some of the numerous factors which may play a part in the distribution of micro-organisms in the upper air. It is generally believed that heavy pre-

TABLE 3*

Flight data

NUMBER	AIR MASSES	WIND, WEATHER	RAINFALL	RELATIVE HUMIDITY	TEMPERATURE	TIME	ALTITUDE (1000 FT.)	BACTERIA	MOLDS	DUST COUNTS
				per cent	°C.	*minutes*				
11. May 23, Wed.	N Pc	W	0.12	72	G 15.4	4	7.6–10	0	8	105
					T −20.3	12	11.5–15.5	1	8	167
		P Cl				11	16–20	5	4	71
						10	19–10	1	5	207
						4	9–3	4	4	255
Control						0		0	0	35
12. May 24, Thur.	N Pc	NW	0	49	G 13.8	9	6–10	5	0	145
					T −29.1	10	11–15	10	3	128
		P Cl				12	16–20	0	3	95
						18	20–23–20	2	1	72
						9	19–10	1	0	162
Control						0		0	0	60
13. May 25, Fri.	N Pc	NE to SW	0.01	72	G 11.5	8	5.5–10	2	1	450
					T −25.9	11	11–16	1	0	327
						13	16.2–20	8	1	185
	P					5	20–21–20	1	0	83
	Ta	O				5	19–10	0	1	430
Control						0		0	0	42
14. May 28, Mon.	Pc	Variable winds	0	60	G 16.4	10	5–10	1	0	84
					T −19.0	8	11–15	1	1	73
						8	16–20–18	0	0	65
		P Cl				8	14–3.5	0	2	87
Control						0		0	0	45
15. May 29, Tues.	N Pa	S	0	72	G 12.8	8	6.7–10	1	2	373
					T −19.6	13	11–15.5	0	0	435
	N Tp					12	16–20	4	0	237
		O				10	20.2–22–20	2	2	145
						6	19–9	2	4	210
Control						0		0	0	29

* See footnote to table 1 for explanation of symbols.

cipitation usually reduces the numbers of micro-organisms in the air. The lethal effects of low temperatures and sunlight on certain types of bacteria have long been recognized. The strength of air currents and their direction, as well as the origin of air

TABLE 4*

Flight data

NUMBER	AIR MASSES	WIND, WEATHER	RAINFALL	RELATIVE HUMIDITY	TEMPERATURE	TIME	ALTITUDE (1000 FT.)	BACTERIA	MOLDS	DUST COUNTS
				per cent	°*C.*	*minutes*				
16. May 31, Thurs.	N Pp	W	0	74	G 21.5	10	4.5-10	7	1	137
					T −17.3	8	11–15	1	1	98
						12	15.5–21–20	2	2	75
		SH				10	19–10	4	1	82
						10	10–3	6	0	115
Control						0		0	0	27
17. Oct. 9, Tues.	Pc and Pp	NW	0	63	G 11.1	5	0.8–3.0	1	5	61
					T − 9.0	6	3.2–6.2	0	3	142
						6	6.5–9.5	2	1	80
		C				4	10–12	0	0	50
						5	12.2–15	10	0	96
						1	15–16	1	5	28
18. Oct. 10, Wed.	N Pc	NW	0	63	G 6.1	8	1–5	2	0	21
					T − 8.8	9	5.5–10	0	0	25
		C				9	10.5–15	2	0	67
						5	14–4	2	0	60
Control						0		0	0	20
19. Oct. 11, Thurs.	N Pc	W ?	0.01	71	G 10	5	0–2.8	1	3	155
					T −10.7	6	3–6	6	3	164
		R				6	6.2–9.2	1	2	502
						5	9.5–12	2	2	342
						6	12.2–15.2	2	3	128
Control						0		0	0	42
20. Oct. 15, Mon.	N Pc	W	0.08	97	G 6.4	3	2–3.5	3	0	150
					T −11.8	4	4–6	2	0	77
		R				4	7–9	1	1	61
						5	9.5–12	2	3	284
						5	12.5–15	2	2	232
Control						0		0	0	15

* See footnote to table 1 for explanation of symbols.

masses are likely to be of significance from the standpoint of the bacterial and fungal populations at the higher altitudes. The humidity of the air may also influence the numbers of bacteria in the atmosphere above the earth. It is highly unlikely that any

TABLE 5*

Flight data

NUMBER	AIR MASSES	WIND, WEATHER	RAINFALL	RELATIVE HUMIDITY	TEMPERATURE	TIME	ALTITUDE (1000 FT.)	BACTERIA	MOLDS	DUST COUNTS
				per cent	*°C.*	*minutes*				
21. Oct. 16, Tues.	Pc	N	0	86	G 6.6	6	2–5	2	0	20
					T −8.5	6	5.2–8.2	0	3	41
						6	8.5–11.5	0	1	18
						4	12–14	1	3	40
		O				4	14.2–16.2	1	0	27
Control						0		0	0	14
22. Oct. 17, Wed.	N Pc	W	0	85	G 8.8	4	1–3	1	4	175
					T −7.0	5	3.5–6	1	2	161
						5	6.5–9	1	0	180
		C				5	9.5–12	3	2	210
						5	13.5–15	0	2	141
Control						0		0	0	62
23. Oct. 19, Fri.	Pc	N	0	85	G 8.6	4	1.7–4.3	12	4	115
					T −12.4	1	5–6	1	0	60
						5	6.4–9	1	0	70
		O				5	9.5–12	1	2	210
						5	12.4–15	6	4	885
Control						0		0	0	45
24. Oct. 25, Thur.	Pa	NNE	0	77	G 7.6	6	2–5	2	1	148
					T −7.3	6	5.2–8.2	0	0	171
						6	8.5–11.5	3	1	170
		C				4	12–14	2	0	181
						4	14.2–16.2	3	0	242
						1	16.5–16.8	1	0	105
25. Oct. 26, Fri.	Pa	SW	0	97	G 8.2	4	1–3	2	1	70
					T −7.3	4	7–9	1	0	37
		Cl				6	10–13	2	1	75
						5	13.5–15	1	0	59
						2	5–3	0	0	47
Control						0		0	0	15

* See footnote to table 1 for explanation of symbols.

combination of these numerous influences is exactly identical on more than one occasion or at any particular altitude.

Reference to the tables indicates that on all of these 30 flights some bacteria were collected which were capable of growing on

TABLE 6*

Flight data

NUMBER	AIR MASSES	WIND, WEATHER	RAINFALL	RELATIVE HUMIDITY	TEMPERATURE	TIME	ALTITUDE (1000 FT.)	BACTERIA	MOLDS	DUST COUNTS
				per cent	°C.	*minutes*				
26. Oct. 30, Tues.	Pc	W	0			6	2–5	1	1	125
						6	5.2–8.2	0	0	288
						5	8.5–11	0	1	81
		C				4	11.2–14.2	0	0	108
						5	14.5–17	1	0	101
Control						0		0	0	40
27. Oct. 31, Wed.		W		69	G 4.7	4	1–3	2	5	152
					T −23.6	5	3.5–6	1	3	140
		P Cl				5	6.5–9	5	1	170
						5	9.4–12.2	1	0	125
						6	12.6–15.8	2	1	432
Control						0		0	0	28
28. Nov. 1, Thur.	N Pc	S	0.08	92	G 11.8	6	2–5	1	1	35
					T −14.4	6	5.2–8.2	2	1	41
	Tg					5	8.5–11	2	0	307
		R				4	11.2–14.2	5	0	833
						5	14.5–17	2	0	310
Control						0		0	0	61
29. Nov. 3, Sat.	Pc	W	0	71	G 1.9	5	2.5–5	0	1	97
					T −17.6	6	5–8	0	0	85
		Cl				6	8–11	1	0	50
						6	11.2–14.2	0	1	232
						7	14.5–18	1	0	215
Control						0		0	0	37
30. Nov. 5, Mon.	N Pp	SW	0.02	87	G 14.5	4	1–3	2	2	56
					T −5.2	7	3.6–6.1	1	0	61
		P Cl				5	6.6–9	1	0	32
						5	9.5–12	0	0	55
						4	13–15	0	0	35
Control						0		0	0	25

* See footnote to table 1 for explanation of symbols.

nutrient agar. In most of the flights bacteria were more numerous than molds, although it must be stressed that the culture medium used was not suitable for the growth of all molds.

Molds were obtained in all the flights except one, no. 18. Seven collections made at altitudes of 20,000 feet or above indicate that bacteria may be found at such altitudes, although the number obtained per minute of collection time (0.26) is lower than that value computed on the basis of all flight collections (0.38). Altitudes are far from being the only factor concerned in the distribution of micro-organisms in the upper air, as is shown in numerous instances when collections at the higher levels during the same flight have resulted in larger numbers of micro-organisms even after the time factors have been adjusted. Flight no. 7 offers an illustration of this nature.

The data obtained in flight no. 4 are of particular interest in view of the fact that on that day an unusual dust storm passed over Boston which was said to have originated in the mid-western drought areas. The bacterial counts were of greater magnitude than those usually observed and would seem to indicate that the increased incidence of bacteria occurred particularly at the lower levels. The mold counts obtained were also higher than in all other flights, with two exceptions. One of these exceptions was flight no. 3 which was conducted at an altitude of 600 to 1000 feet for sixteen minutes over a wooded region in Waltham, Massachusetts. The dust counts observed on that date were also higher than have been experienced in any other flight.

TYPES OF BACTERIA ISOLATED

Transfers from colonies on plates were made of characteristic micro-organisms which were obtained from collections made at altitudes above 10,000 feet. Bacterial cultures of this origin were subjected to the common dilution plate methods to obtain pure cultures, which were subsequently examined by the biochemical, physiological and morphological means necessary for identification. The following bacteria were identified in accordance with the characteristics cited in Bergey's *Manual of Determinative Bacteriology*, 4th edition, 1934.

Bacillaceae
Bacillus aerosporus
Bacillus albolactis
Bacillus cereus
Bacillus evanidus
Bacillus Freudenreichii
Bacillus ruminatus
Bacillus simplex
Bacillus teres
Bacillus vulgatus

Bacteriaceae
Achromobacter gasoformans
Achromobacter Pikowskyi
Achromobacter reticularum
Achromobacter stoloniferum
Kurthia Zopfii

Coccaceae
Staphylococcus albus
Staphylococcus citreus
Micrococcus candidans
Micrococcus flavus
Micrococcus ureae
Micrococcus varians

TYPES OF MOLDS ISOLATED

A number of characteristic mold colonies obtained above 10-000 feet were examined morphologically after transfer and cultivation on Czapek's agar.

Aspergilli and Penicillia were the more common molds found; 12 cultures of the former and 6 of the latter were obtained from these collections.

Molds of the following genera have also been identified from this series of flights; *Rhizopus, Mucor, Oöspora, Monosporium, Macrosporium, Tilachlidium, Fusarium.*

Actinomyces

Seven cultures of *Actinomyces* were isolated in the same collections.

No yeasts were found, although in certain flights previous to this group, yeasts had been obtained.

Pollen

Specimens of pollen were obtained in 3 flights, one of which was at an altitude of over 20,000 feet.

Dust

The dust counts show rather wide variations on various flights and at different altitudes. It is worthy of note that the dust counts are of much greater magnitude than those obtained for micro-organisms. Little is known concerning the origin of this dust, although the logical assumption is that it comes from the earth. A statistical analysis was made of the dust counts obtained on various days of the week in order to make comparisons of those collections above 10,000 feet with others taken below that level, to observe any possible relation between industrial activity and dust counts. No definite relations of this nature have been determined as yet.

The dust counts on the controls are due to the fact that the disks used as collectors are not free of dust in spite of the fact that they are washed with chloroform previous to use. More extensive preliminary washing after the first few flights was found to lower the control counts in this series.

Precautions were taken to insure the fact no dust was getting into the collector from the exhaust of the airplane motor and are cited in a previous report (Proctor, 1934).

DISCUSSION

The results of these observations concerning the microbiology of the upper air, as shown by the collections made, indicate a variable population, both from the standpoint of bacterial and mold types and numbers. The ability of these living microorganisms to attain altitudes of 20,000 feet or more through the chance action of air currents is particularly significant as it suggests the almost limitless possibilities of travel in a horizontal direction. The survival of such forms despite the many influences which are unfavorable to their existence is also significant in view of the length of time for which they may remain viable.

Those micro-organisms which have been identified to date are largely types associated with soil, air and water. Some of the molds found were members of genera which contain plant pathogens but no recognized pathogens have been identified as yet. There is no valid reason to believe that such micro-organisms are not subject to equally extensive flights, however.

The presence of pollen at high altitudes also indicates the importance of air as a vehicle for the transmission of wind-borne pollens over wide areas.

The high dust counts obtained in comparison to the numbers of micro-organisms is interesting in view of the various possible sources of dust, some of which, as from soil, might be also associated with high bacterial counts, while in other cases particles from smoke stacks and industries might be sterile. An investigation particularly directed towards the origin and composition of such particles has recently been initiated.

CONCLUSIONS

The upper air, which is constantly changing with respect to many physical factors, is at the same time a vehicle for the dispersion and transmission of many types of micro-organisms, pollens and dust particles.

The extent of vertical movement of such organisms has been demonstrated to be at least several miles.

The types and numbers of micro-organisms found, which were largely types associated with soil and water, are subject to wide variation. These variations are due to numerous influences which are constantly interacting in the upper air strata, to the varied sources of the micro-organisms and to the ability of these micro-organisms to survive unfavorable conditions.

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