Bacteria in Antarctica*

Various Species Found in Snow and Ice, and Their Origin

By A. L. McLean

THE researches we were able to prosecute during Sir Douglas Mawson's Australasian Antarctic Expedition (1911-14) in the subject of bacterial flora of snow and ice have given rise to certain queries which, if accurately answered and correlated to the work of four previous observers, should go far towards an elucidation of the bacteriology of Antarctica as a whole.

Dr. Ekelöf[1], whose investigation for nearly two years of the soil of Snow Hill Island, near Graham Land, were rich in results and of great scientific value, made experimental exposures of Petri plates for possible bacteria in the air. He found positive growths on at least half of his culture media, claiming that a Petri plate had to be exposed for two hours for one bacterium to settle on it. His conclusion is, on the evidence of examinations of soil and on account of the unprecedented weather conditions of his Antarctic station, that the organisms he obtained from the air were impurities carried into it by the wind from the soil.

Dr. Gazert,[2] when frozen in the pack-ice to the north of Kaiser Wilhelm II. Land, sought for bacteria in the atmosphere by making cultures of freshly fallen snow. The cultures were found in every instance to be sterile.

Dr. Pirie,[3] during his voyage in the Weddell Sea, exposed plates and tubes in the crow's nest (at the top of the mainmast) of the *Scotia*, at the longest for twenty hours, with negative results. During the winter months at Scotia Bay he was unsuccessful in similar experiments, as also during the summer. He records, too, that plates of agar and media (for denitrifying organisms) were exposed on top of the deck-laboratory during the voyage in the Weddell Sea in 1903. He considered the last-named cultures to be unsatisfactory, owing to the possibility of contamination from the ship and from spray. "Growths of (apparently) *Staphylococcus pyogenes albus* and of a yellow coccus, possibly *Staphylococcus pyogenes citreus*, were obtained, and also denitrifying organisms."

With this evidence before us it is instructive to learn that Dr. Atkinson, of Capt. Scott's British Antarctic Expedition (1910-13), apparently made bacteriological examinations of snow.[4] "Atkinson is pretty certain that he has isolated a very motile bacterium in the snow. It is probably air-borne, and, though no bateria have been found in the air, this may be carried in upper currents and brought down by the snow. If correct, it is an interesting discovery."

Lastly, so far back as 1893, it is the record of Nansen in "Farthest North" that he made frequent microscopic examinations during the second summer of fresh-water pools on the floe-ice of the North Polar basin. Algæ and diatoms were proved to germinate at the bottom of these pools, providing the food material of infusoria and flagellata. Bacteria, he says, were occasionally observed. Again, Nansen noticed that in places the surface of the snow was sprinkled with dust, and he was led, after more extended inquiries, to regard the phenomenon as universal over the North Polar Sea. He attributes this fact to floating dust being carried by lofty air-currents from southern lands and then descending to the surface in falling snow.

Doubtless, too, one may infer that equatorial air-currents at a high altitude convey myriads of dust-motes towards the South Pole, where they descend, free or clinging to snow-particles, over the great ice-capped continent of Antarctica. And as evidence towards the probable truth of this speculation we have been able to furnish some isolated observations.

The locus of the main base of the Australasian Antarctic Expedition in Adelie Land was singularly fitted for research of a general character on ice and snow, since here the great inland plateau undulates downwards in *névé-fields*, declining gradually for hundreds of miles, to fall abruptly in glacial slopes to the sea. In fact, we were on the verge of the continent, with no naked mountains or outcropping nunataks[5] encircling us to the south.

[The results which were obtained from an examination of frozen algæ and frozen seaweed led us to inquire further into the bacterial content of the glacier-ice —apparently as pure as distilled water! And so the organic content of frozen algæ makes a suitable point of departure in considerations of a general character, for in these dirty green lumps of ice are represented practically the whole of the low life which exists and actively multiplies in Antarctica: algæe, diatoms (unicellular algæ), protozoa, rotifera, and bacteria. The algæ (including the diatoms) are universally found, according to the scientific reports of other Antarctic expeditions, as marine or fresh-water types in the ice-girt zone surrounding the continent. In Adelie Land one became accustomed to note in the summer-time that certain of the thawed pools among the rocky ridges were filled with a greenish slime—the filamentous, multicellular algæ.]

On comparing results in Adelie Land and in Australia, it is evident that at least four species of bacteria exist in the frozen algæ:—

(1) Gram-positive cocci, with fine, white colonies, liquefying gelatine very slowly, were almost invariably obtained in cultures.

(2) Agram-positive, sporing bacillus spreading as an abundant, pale, wrinkled, and adherent growth on all media.

(3) Gram-positive, chained, sporing bacilli, occurring as a white, profuse growth on all media. In cover-slip preparations of the ice chained bacilli were always seen.

(4) Short gram-positive bacilli, showing on agar a milky-white growth, which afterwards became yellowish in tint.

The fact of the mere presence of bacterial life in frozen algae would not seem remarkable along the fringe of the continent, where lichens and mosses thrive during the short periods of warmer weather, and where there is a continuous accession of low life from the sea, the soil, and animals. It is only natural to expect them, and to infer, further, that they migrate for a variable distance into the all-enveloping mass of ice and snow, to all intents and purposes free from organic life.

Again, in morainic ice—macroscopically pure but for particles of soil and grit in small amount—protozoa-like organisms were present, and in several cultures appeared fine, white colonies of gram-positive, staphylococci, together with the gram-positive, sporing bacilli of the white, wrinkled, adherent growth already described.

When our observations had arrived at this juncture there was a clear indication to go further afield in the examination of the ice; at all events, to see the extent of the local bacterial flora. So specimens were procured from various points, free from obvious contamination, on the ascending glacier.

(1) In the magnetic cave, cut shaft-like through the slope of blue ice, about 1100 yards south of the Hut, at an altitude of 300 ft. above the sea, were found in cultures cocci and diplococci, slender bacilli, and a "yeast." Protozoan organisms were also seen.

(2) In cover-slip preparations 200 to 300 yards, 500 yards, and 1000 yards south of the Hut occurred cocci, motile bacilli, yeast-like bodies, and protozoa.

(3) The surface-ice at 1100 yards, altitude 300 ft., yielded in cultures cocci (staphylococci) and short stout bacilli.

(4) At one mile, altitude 600 ft. to 700 ft., in surface-ice, appeared in cultures gram-positive staphylococci and slender, gram-negative, chained bacilli. Protozoa and yeast-like bodies were demonstrated in the thawed ice-chips.

(5) In the vicinity of Aladdin's Cave, five miles south of the Hut, and at an altitude of 1500 ft., surface-ice showed the presence of protozoa and yeast-like bodies. Gram-positive cocci grew in cultures on several occasions.

Ice at a depth of 4 ft. contained, besides protozoa and yeast-like bodies, gram-positive cocci and gram-negative bacilli, all in smaller numbers than on the surface. Nothing was obtained in a few cultures.

In ice at 7 ft.—from the wall of the cave—cultures were more successful, demonstrating gram-positive cocci and gram-negative bacilli (probably cocco-bacilli). Protozoans and yeast-like bodies were also present.

(6) From the Cathedral Grotto—at eleven miles, and at an altitude of 1800 ft. above the sea—specimens of ice gave in cultures growths of a gram-positive coccus and a gram-negative cocco-bacillus. No protozoa or yeast-like bodies were observed in the preparation from thawed ice.

(7) In a position fifty miles west of the Hut and twenty-five miles inland, nearly 4000 ft, high on the plateau, surface *névé* (a transition between snow and ice) was found to contain cocci and bacilli in their usual numbers, but no protozoa or yeast-like bodies were seen. Many of the bacilli were clumped in zooglœa mases. From four original cultures and several subcultures were isolated gram-positive cocci and gram-negative cocco-bacilli, similar to those grown from other specimens of glacier-ice.

Then, too, we should adduce the evidence of the cultures made in Antarctic and carried back to Australia for examination.

On a rare calm day early in January, 1914, six agar tubes were taken, with a spirit-lamp and platinum needle, up the slope of the glacier nearly half a mile towards the south-east, where the glacier could not possibly have been soiled by the many sledging parties which passed up and down during the summer.

There was no opportunity at the time to go further afield. The sun was bright and warm, there was no wind, and the ice was covered with a humid sheen of moisture. The tubes were inoculated from loops of liquid collected with the needle in small cups where thaw-water had accumulated. They were then carried back to the ship and placed in an incubator, which ran at a temperature varying, during blizzards, from about 10° to 15° C.; as a general rule, the temperature was between 18° and 20° C.

Dr. Cleland's report shows that nine cultures of ice were received, that of these, three showed no colonies, and were discarded, and that the remaining six on agar slopes exhibited growth. From three tubes "yeasts" were isolated, two of them giving a pink growth on agar, the remaining one a creamy-yellow growth. Two cultures showed the presence of a gram-positive coccus, producing a fine growth, which died out in subsequent subcultures.

It is a curious fact, and yet a well-known experience, to find that bacteria may live dormant in ice for prolonged periods, and that infection may be carried through ice, but it is not so generally recognized that some bacteria prefer to grow on ice. Micro-organisms, as a rule, are capable of resisting a low temperature when their ordinary activities cease, and they tend, either as single units or in clusters, to throw out a mucilaginous protein substance for their protection. Ravenel, Macfadyen, and Rowland have demonstrated that several bacilli will bear exposure for a few days to the temperature of liquid air (—192° C. to —183° C.). More recently it has been proved that certain bacteria actually survive the temperature of liquid hydrogen (—252° C.), applied for so long a period as ten hours. Bearing in mind such experiments conducted *in vitro*, we could understand that certain organisms carried by dust-motes to the vicinity of the south geographical pole (at an altitude of approximately 10,000 ft.) could retain their vitality in a temperature of —100° C. (—148° F.), if ever the midwinter temperature descends to such a low limit. Certainly, in the prolonged insolation of the summer-time, some hardy organisms on the surface could thaw out, become free, and increase in numbers.

On the other hand, bacteria and their spores have almost a defined limit of resistance to heat—57° C., if applied long enough. Some germs are thermophilic, mainly those which live and multiply in warm-blooded animals; while others—in general terms, the bacteria of the sea, the soil, and the air—prefer the mean temperature of their environment.

In the Antarctic—and the same hold goods of the Arctic regions—there is a definite fauna, comprising in the former case the various species of seals, whales, and birds and their parasites, insect-like mites of the mosses, rotifera, and a fairly prolific marine life. The flora of the south is summed up in the lichens, mosses, and algæ, the last-named having a vast distribution amongst the ice encircling and adhering to the continent. Primordial, lowest of all, and standing as an evolutionary basis of the animal and vegetable kingdom

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* From *Nature*.

[1] "Bakteriologische Studien wahrend der Schwedischen Sudpolar-Expedition (1901-3)." (Stockholm, 1908.)

[2] Deutsche Sudpolar-Expedition, 1901-3. "Untersuchungen uber Meeresbakterien und ihren Einfluss auf den Stoffwechsel in Meere."

[3] "Notes on Antarctic Bacteriology." (Edinburgh, 1912.)

[4] "Scott's Last Expedition," vol. i., p. 211. (1913.) We have been unable so far to confer with Dr. Atkinson with reference to his actual results and general conclusions.

[5] The Western Party, under Mr. F. H. Bickerton, discovered a small piece of rock on the snow at a height of 3,000 ft., 17 miles southwest of the Hut in Adelie Land. This was afterwards identified in Melbourne by Prof. Skeats and Mr. Stillwell as a meteorite.

are the bacteria, which we may presume to say are universal—clinging to the myriad dust-motes which float from the north; descending in snow on the Antarctic plateau; paralyzed for long winter months; active and acclimatized in the liquid thaw of summer; segmenting or sporing in their multiplication; dormant again in the inter-crystalline canaliculi of the *névé* and ice, and free once more to live and increase in the viable reticulum of the glacier. Such a speculative theory may be the key to their cycle of life in Antarctica.

Liquid containing salts in solution does not completely freeze at a temperature of 0° C. (32° F.), and this factor is very important in the maintenance of low and higher forms of Antarctic life. The late Mr. James Murphy[6], of Sir Ernest Shackleton's British Antarctic Expedition (1907-9), has contributed some unique evidence of the habits and powers of resistance to cold exhibited by the rotifers and water-bears.

"To test the degree of cold which they could stand, blocks of ice were cut from the lakes (saline) and exposed to the air in the coldest weather of the whole winter. By boring into the center of the blocks we found that they were as cold as the air. A temperature of —40° F. did not kill the animals.

"Then they were alternately frozen and thawed weekly for a long period and took no harm. They were dried and frozen, and thawed and moistened, and still they lived. At last they were dried, and the bottle containing them was immersed in boiling water, which was allowed to cool gradually, and still a great number survived. . . .

"Such is the vitality of these little animals that they can endure being taken from ice at a *minus* temperature, thawed, dried, and subjected to a temperature not very far short of boiling-point, all within a few hours (and a range of more than 200° F.). . . ."

It would seem that bacteria were the ideal denizens of an environment where, for the greater part of the year, all visible life is banished, and where their minute size, protective changes of form, and versatile reaction to moisture, low temperature, and concentration of salts would be most advantageous for existence. The bacteria caught up in the frozen sea within the liquid sludge of cryohydrates, which circulates between the crystals of fresh-water ice, learn to live, and probably multiply, in a medium of much higher concentration than the ocean to which they are accustomed.

The question now seems naturally to arise: How are we to explain the existence and multiplication of bacteria in ice? And to satisfy such a query we should endeavor to discover what is the ultimate composition of ice, how the crystals of ice are inter-related, and what are the intimate changes which occur in a descending or rising temperature.

We refer to Mr. J. Y. Buchanan[7], formerly of the *Challenger* Expedition (1874), for the most modern views of ice-formation.

As a result of many exhaustive experiments on the changes which occur in freezing non-saturated saline solutions, he finds that the crystals formed by freezing a saline solution are in their ultimate constitution free from salt. That is to say that "the crystals formed in freezing a non-saturated saline solution are pure ice, and that the salt from which they cannot be freed does belong to the adhering brine." Therefore, we may imagine that when sea-water freezes the primary solidification which takes place is of the fresh-water content, the salts in solution being rejected into the channels which now exist between the pure crystals. As the temperature is still further reduced, accretions of pure ice go to the crystals, and the brine, still further concentrated, remains in the channeled meshwork.

In the case of the glacier-ice of Adelie Land, which we wish particularly to consider, one would expect the ice to be very pure; in fact, the superimposed layers formed from the snow which has fallen should be, presumably, as fresh as distilled water. But assuming, as we do, that a large amount of aerial dust is distributed over the South Polar plateau, and that atmospheric gases are combined with the snow, the ice contains mineral constituents, without doubt, in much more dilute solution than is present in the rain-water of a more temperate climate. And, considering that this contamination by dust-motes has gone on for countless æons, the whole thickness of the polar ice-cap is impregnated with minute foreign bodies.

On dissecting a piece of the glacier we find that a disintegration of the interlocking grains, similar to that which occurs in upturned slabs of sea-ice, takes place on its exposure to the warmth of the sun or to a temperature just below the freezing-point of fresh water. As Buchanan says: "Under the influence of the sun's rays the binding material melts first, the continuity of the block is destroyed, the individual grains become loose and rattle if the block be shaken, and finally they fall into a heap. A block of glacier-ice is a geometrical curiosity. It consists of a number of solid bodies of different sizes and of quite irregular shapes, yet they fit into each other as exactly and fill space as completely as could the cubes referred to above."

Buchanan made his studies of ice on the Alpine glaciers, which, in comparison with the ice-sheet of Antarctica, move rapidly, and, of course, are grossly contaminated by soil, rock and dust. Still, one of the first phenomena we remarked when stepping on to the ice-foot at Cape Denison, Adelie Land, was the large amount of granular rubble which formed the surface of the glacier. In other words, the summer sun had thawed out all the cementing channels, and the crystals lay melting in a clear slush of liquid.

To a living organism a few micro-millimetres in length, a block of glacier-ice not completely solidified would be a veritable labyrinth of minute tunnels filled with liquid containing salts in solution. In every direction the tunnels would be viable, so that a single bacterium might easily pass from top to bottom of the block. The same lump, as an integral part of the glacier, would still be perforated with devious and circuitous passages, inosculating with others in the surrounding ice, but the watery contents of these passages would follow laws of movement dependent upon gravity, the slope and movement of the glacier, the presence of small seams and cracks in the ice, and the gradient of temperature from above downwards.

Sufficient has been said to indicate that if in the section of ice we are considering the temperature approaches to freezing-point, the channels of adhering fluid which encircle the crystals would permeate the glacier down to a definite point where, if the mean annual temperature were low enough, the ice would be solid and impervious. We are led to suppose from Buchanan's observations that the critical temperature of solidification may be as low as —13° C., though in Antarctica, where the ice is purer, it should be 4° or 5° higher. Granting that such a temperature may be several degrees from the actual truth, we may at least be sure that for 5° below the freezing-point of fresh water the glacier-ice of Antarctica is pervious to bacteria, and contains a medium suitable for their reproduction.

In Adelie Land the mean annual temperature at sea-level lies between —15° and —20° C., but on mounting the plateau which falls steeply to the coast, the temperature descends at the rate of almost 4° for every 1000 feet. In the summer-time the shade temperature registered on several occasions 5.5° C. (40° F.), and for three months at least the temperature, except for unusual fluctuations due to blizzards, never fell much below —10° C., and was very often close to 0° C. Considering, too, that there is a very appreciable amount of sunshine between the equinoxes, the period during which bacterial life and growth would be possible might be extended, during a favorable summer, up to four months. The action of sunlight is of paramount importance in promoting a thaw throughout the ice canaliculi, especially when we remember that the shade temperature may register 0° C. at the same time as the thermometer in the sun rises to 16° C.

The important point at issue is that the northern slopes of the glacier fall towards the sea at such an angle that the rays of the sun for some months during the summer are normal to the surface, thereby increasing the intra-glacial thaw, and for short periods causing the temperature of the whole mass in the lower latitudes to rise within a few degrees of freezing-point, the optimum temperature of the micro-organisms of ice and snow. At the south geographical pole, elevated to 10,000 feet, the obliquity of the sun's rays and the low temperature would not encourage bacterial life except in the surface layers of snow, and that only for a few weeks at the summer solstice. Assuming that the greater part of the continent is at a more or less uniform height of 6000 feet, we should conclude that the organisms which descend from the air are, when buried to a certain depth, wholly deprived of a free-swimming existence, until in the plentitude of ages they arrive at that northern boundary where the summer thaw begins.

It will be apposite now to review the few observations which were made on snow before passing to a few remarks on the meteorology of the southern hemisphere.

(1) Gram-positive cocci and gram-negative, sporing bacilli grew in culture from snow of a sastruga or snow-wave one-third of a mile southeast of the Hut.

(2) On three occasions when falling snow was gathered in a sterile basin, elaborate precautions having been taken to prevent contamination, the thawed-out samples showed under a coverslip cocci, motile bacilli, and, invariably, zoogloea masses of bacteria in moderate numbers. Diplococci, and occasionally cocci, were observed to be invested by a pale capsule. In one case doubtful organic matter in the form of vegetable cells was noted.

(3) A *glucose agar slope* culture of falling snow showed a few small greyish colonies, which were not examined.

Slender as these results are, they become of more importance when correlated with the many positive findings made in glacier-ice—the vast repository of the falling snow. They are meaningless, too, unless we consider the probable origin of the bacteria which cling to the crystals of snow.

Regarded simply, the circulation of air in the southern hemisphere has certain main characteristics—a widespread uprush from equatorial, tropic and sub-tropic zones; a continuous flow at a high level towards the southern continent; a subsidence of successive layers of cool air, increasing in density and coincident with a rising barometric pressure; a concentration of air at high barometric pressure over the vast crown of lofty Antarctica; a relief of pressure in the torrential bursts of blizzards through to the low-pressure belt of the Southern Ocean, and, in wide terms, the genesis of a low equatorial return current modified and deviated by such factors as earth-movement, latitude, disposition of island, sea, and continent, and configuration of the land.

Bacteria or their spores may be found in the atmosphere free, incorporated with minute particles of aqueous vapor, or clinging to small foreign bodies. With these foreign bodies or dust-motes we know that they ascend under the impetus of rising equatorial air into the atmosphere to a considerable height, until at length they come under the influence of the great poleward-flowing current. The bacteria meanwhile have cooled, become paralyzed, and, either singly or in segregated masses, thrown out their protective capsule of protein material. They travel to the Pole, and here are frozen to spicules of ice or with the dust which has conveyed them are attached to crystalline snowflakes, sinking lower with the descending strata of air, and alighting at last on the surface of the plateau.

And now, sparse or in numbers, the frozen organisms, extruded with the dust-mote they accompanied to the periphery of the nuclear snow-crystal, commence a new life-history.

When the snowflakes—on the plateau of Antarctica snow is mostly in the form of sago-like granules—have recently fallen, they lie together in soft, downy, flocculent heaps enclosing, in proportion to the space they occupy, a large volume of air. Under the influence of gravity and the pressure of the wind, and in dependence, too, on the temperature and humidity of the air, the snow becomes denser and more compact, the enclosed air is expelled, and the snow-crystals increase in size. Thus we may conceive that the bacteria tend to be expelled into the interstices between separate crystals, where they await the time when the temperature will rise sufficiently to provide a liquid medium in which their life and species may be renewed. If the temperature still remains too low for liquefaction of the comparatively impure snow adhering around the primary pure crystal, the slow metamorphosis of the snow into *névé* goes on under more or less dry conditions.

In conclusion, if we trace out briefly the subsequent history of these bacteria of ice and snow, we see them in the slow northward surge of the glacier set floating in ice-tongues and bergs of the Antarctic Ocean, where they gradually thaw out and probably become accustomed to the salinity of the sea. They circulate throughout the immense volume of water, clinging to the plankton of the surface, traveling to various depths, reaching, maybe, the ooze in company with sinking foreign bodies. They migrate in the vast, moving ocean currents towards northern lands, where some remain as marine bacteria; others enter the mouths of rivers and become adapted to life in the fresh-water medium they knew in Antarctica, while still others are stranded on the littoral, whence, in a dry condition, they may be transported by wind to a new soil, assuming, perhaps, the characters of anaerobic bacteria. The cycle—centuries or geological periods in duration—begins once more when, in a temperate zone, the descendants, by an endless gamut of fusion or sporulation of the original organisms, rise on dust-motes and rejoin again the bacteria of the upper air, once more liable to enter the current flowing continuously towards the southern pole of the earth.

[6] "The Heart of the Antarctic." By Sir E. H. Shackleton, C.V.O. Vol. ii., p. 238. (London, 1909.)

[7] "Ice and its Natural History."