

AUSTRALASIAN ANTARCTIC EXPEDITION

1911-14.

UNDER THE LEADERSHIP OF SIR DOUGLAS MAWSON, D.Sc., B.E.

---

SCIENTIFIC REPORTS.

SERIES C.—ZOOLOGY AND BOTANY.

VOL. VII. PART 4.

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BACTERIOLOGICAL AND OTHER  
RESEARCHES

BY

A. L. McLEAN, B.A., M.D., CH.M. (M.C.).

WITH ELEVEN PLATES AND THIRTEEN TEXT-FIGURES.

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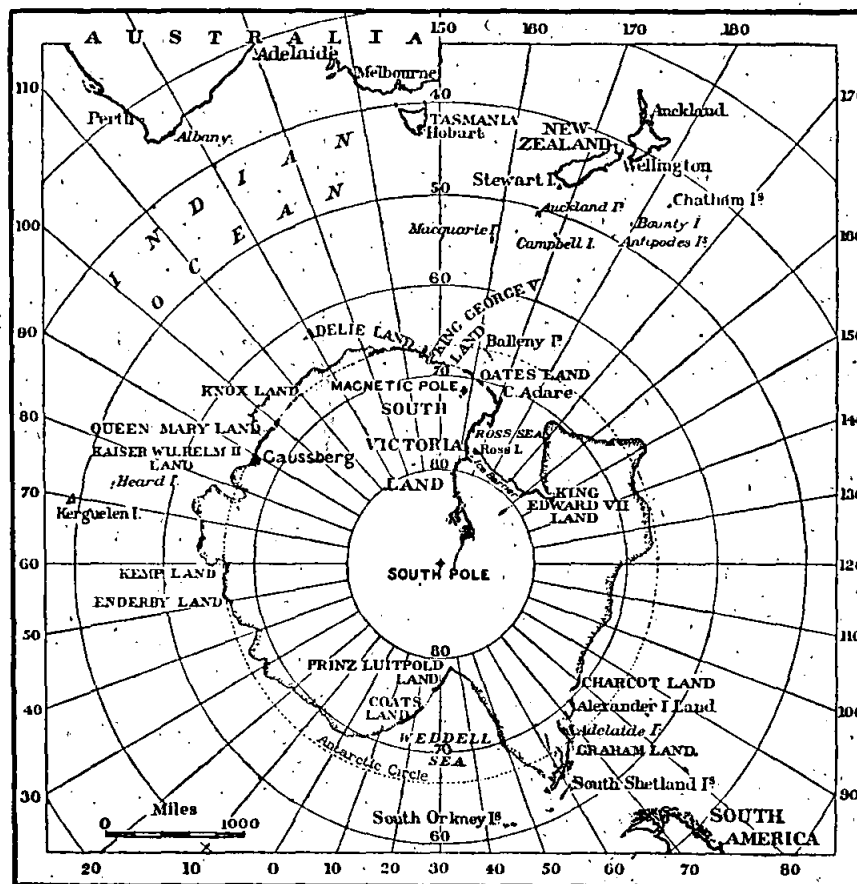
# BACTERIOLOGICAL AND OTHER RESEARCHES.

By ARCHIBALD LANG McLEAN, B.A., M.D., Ch.M. (M.C.), Surgeon and Bacteriologist.

With Eleven Plates and Thirteen Text-figures.

## INTRODUCTION.

THE object of the Australasian Antarctic Expedition (1911-1914) was to explore and to prosecute scientific investigations over a tract of 2,000 miles of coast line contained within the Australian Quadrant of Antarctica (between  $90^{\circ}$  and  $180^{\circ}$  east longitude). This area of the continent of Antarctica lies to the south of Australia and New Zealand.



A Map of the Antarctic Regions as known at the present day.

Moreover, it was fitting that an expedition composed of men of the Commonwealth and Dominion should have attempted to define its geographical contours, especially as these had, up to the year 1911, only been vaguely indicated. Two navigators, Dumont D'Urville and Charles Wilkes, in the year 1840, proved the existence of

undoubted landfalls in isolated localities, but no one had actually wintered on the mainland nor had made any exhaustive scientific enquiries between Cape Adare on the east and Gaussberg to the west. With such an outlook, it may be understood that the scientific aims of the Australasian Expedition were directed so as to be as wide and embracing as possible.

Bacteriology was to be my special department, and the task of obtaining various suggestions as to lines of work and securing suitable apparatus was compressed into two short months towards the end of 1911.

The field was one in which four previous bacteriologists had carried out important researches, to whose records I had access only in one instance, owing to the exigencies of time. In any case, a great deal had been accomplished, but in this new sphere of Antarctic land every fresh observation was of value, in so far as it constituted an addition to the sum of scientific knowledge.

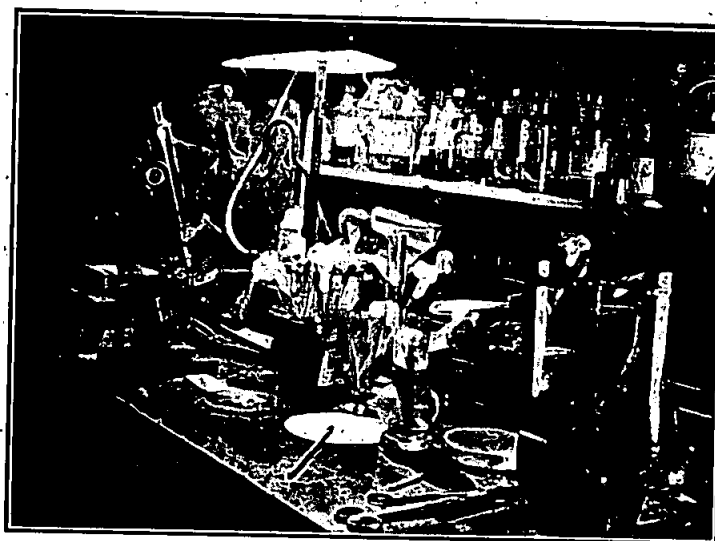
Specific suggestions were not wanting. Each day new ideas were discussed, and the programme increased in size until it became difficult to select what would be most practicable and fertile in result. Antarctica was, so to speak, another world, the peculiar conditions of which were worthy of study in the records of exploration. The tools for the task were to be suited to the environment. Presumably the South was germ-free, but the intestines of antarctic animals must have some kind of a bacterial flora. The birds and seals were possessed of scientific interest, even for a student in medicine. The healthiness of the explorer's life was proverbial—should not one be in a position to study physiology, immunity and psychology under unique circumstances? Such were some of the general queries, and the actual practice has narrowed down to the chapters which follow.

The equipment I took to Adelie Land was multifarious in detail, on the assumption that it is better to overstock than to fail in an important observation through the lack of some simple instrument or chemical. There was the problem of space, of course, and it was a vital one on our small exploring ship, laden with provisions for thirty-two men over a period of two years, four hundred tons of coal, sledges, the timber of four huts, two wireless plants, an aeroplane, a motor-boat, sledging dogs, scientific instruments, and a great miscellany of articles either lashed on deck or stored below. Such a recital will bring home the essentially practical side of exploration.

It was useless to attempt any bacteriological work on the passage to Adelie Land amidst the impedimenta which filled the cabins and overflowed on to the decks; a disadvantage which I felt on arriving at Macquarie Island, with its wonderful fauna and diverse soil characters. The first necessity was to establish our bases at various points, making the random notes or collections which time permitted.

In Adelie Land a hut was built in a few weeks, and here, after a time, I was able gradually to unpack my boxes dumped with the cargo on to the ice-foot; afterwards to find space for some of their contents in the dwelling which formed our Main Base in Antarctica for a period of two years.

The heat for the incubator was supplied by a kerosene lamp. The ether capsule and lever regulators worked well, so that it was possible to grow cultures either at  $18^{\circ}$  to  $20^{\circ}$  C., or at  $37^{\circ}$  C. It would have been a great advantage to have had two incubators for this purpose; especially during the final relief cruise of the "Aurora," when cultures from animals were grown with others from soil, marine mud, and ice. As the latter were grown at  $18^{\circ}$  to  $20^{\circ}$  C., many of the former either did not develop or soon ceased to grow. The rolling of the ship during gales and blizzards—as well as the occasional extremities of cold—made it difficult to keep the incubator at a uniform temperature.



Bacteriological Laboratory in the Hut at Adelie Land.

A large stock of culture media—gelatine, nutrient agar, serum agar and glucose agar—were carried in tins, each containing one gross of tubes. It would have been possible to manufacture media on the spot—and for this purpose the necessary materials were taken—but subsequently it appeared that the Hut was infested by the spores of moulds which would doubtless have infected the culture tubes. Even though the media became frozen soon after the ship had entered the pack-ice, two tins were found, after a few months in the Hut, to have become contaminated, and were discarded, rendering it necessary, in using a doubtful tube, to incubate it beforehand for a period of twenty-four or forty-eight hours. Dr. Ekelöf, of the Swedish Expedition (1901-1903), and Dr. Pirie, of the Scottish Expedition (1902-1904), record similar experiences.

Again, the gelatine media, after being frozen, were found to split and crack after inoculation by stab-culture. Sometimes the whole medium became broken up, and the conditions for anaerobic growth and gas-formation were not present. Actually

the number of tubes available for making cultures dwindled so much, that I was not able always to make subcultures, and the bacteriological work had to be limited.

There were many difficulties which presented themselves and had severally to be overcome or recognised as insuperable. Tests which could be quickly and accurately carried out in an ordinary laboratory, replete with conveniences, occupied a much longer time in the winter quarters at Cape Denison. The claims of general work, incidental to the operations of a self-contained expedition, were always present, though I should mention that our leader, Sir Douglas Mawson, gave to all those engaged in special research the utmost encouragement.

A small corner of the Hut was reserved for bacteriology. Here, a few shelves and a table accommodated stains and other reagents, slides, a spirit lamp, a centrifuge, a microscope, a steriliser, and other miscellaneous apparatus. For more than four months I was unable to make up Gram's iodine, owing to the potassium iodide and iodine having been misplaced. Smears from many of the cultures made from animals in 1912 were, therefore, not stained by Gram's method. The boxes containing my stock of materials were buried in snow outside the Hut and were only accessible on the rare fine days, when they had to be dug out, opened and re-packed.

The Main Base of the Expedition was located at Commonwealth Bay, Adelie Land. Here, at Cape Denison, is an outcrop of rocks, mainly gneissic granite, forming about 1 mile of the coast-line, from east to west, and not more than one-third of a mile at the widest part, measured from the sea back to the moraine in which the glacier ends to the south. On either side streams down the piedmont glacier, presenting at the sea-front a perpendicular wall of ice, from 80 to 120 feet in height. Five miles to the west, following the icy face, is another outcrop, where a large rookery of Antarctic petrels was subsequently discovered. The glacier's wall continues then for some 20 miles to the next outcrop. From observations made by sledging parties, and as a result of the three Antarctic cruises of the "Aurora," it is probable that these protrusions of the underlying land occur at irregular intervals along the glaciated coast of the continent for 1,300 miles to Queen Mary Land, where the second Antarctic party landed, under the leadership of Mr. Frank Wild. Dr. S. E. Jones, the surgeon, was equipped to carry on bacteriological work, but was forced to abandon any researches on account of his culture media becoming contaminated.

The weather in Adelie Land was extraordinarily severe. An almost continuous blizzard blows there year after year. The average hourly velocity of this torrent of polar air amounts to 50 miles per hour, a record for any inhabited locality on the face of the earth. On our arrival at Commonwealth Bay, there were seals and penguins, skua gulls and petrels to be found along the foreshores of Cape Denison, but we were all so busied in building huts, erecting wireless masts, and making the winter quarters habitable, that I had scant opportunity until the following spring of securing cultures.

from animals with any degree of leisure. In the summer of 1912 to 1913, most of us were away on long sledging journeys, and all the collections were packed up in readiness for our return to Australia. So, it may be understood that, during the winter of 1912, most attention was directed to the bacteriology of ice, snow, and soil.

Owing to the calamity which befel one of the sledging parties, seven men were obliged to spend another winter in Adelie Land. During this period I was unable to continue my special studies, as the incubator had been taken on board the "Aurora," and had not been transferred again to the Hut owing to the unprecedented weather. But there was ample occupation adding to the biological collections, while our domestic duties and the regular meteorological and magnetic observations kept us all fully employed during 1913. However, when the ship returned to relieve us in December, 1913, I found that the incubator had been returned with a fresh consignment of culture tubes. For a week, then, during a spell of summer calm, I was able to make certain cultures which were carried back to Australia, and on which reports, appearing in due course, were furnished by the Bureau of Microbiology, Sydney.

Finally, there are certain acknowledgments I should like to make; in particular, the advice and practical help given me by Dr. J. B. Cleland. To him and Drs. E. W. Ferguson and K. Smith I am indebted for the investigation of cultures and specimens brought back from Antarctica. Professor D. A. Welsh of Sydney University offered me many suggestions and apparatus which proved invaluable in the South. While in Hobart, I was grateful to Dr. J. S. Purdy for the use of the Board of Health laboratory. In Adelie Land, one was generally in touch with, and ready to aid the other's special department of science; I should mention the help of my comrades, Messrs. J. G. Hunter and W. H. Hannam. Later, in Sydney, Dr. B. T. Edye assisted me to standardise two instruments, and Mr. R. Grant of the Bureau of Microbiology kindly took the micro-photographs which appear among the illustrations. Dr. O. Latham, pathologist of the Department of Lunacy, was unfailing in his sympathetic assistance. Six photographs by Mr. J. F. Hurley appear among the plates at the end of the Report. They represent artistically the surroundings of the Hut at Cape Denison.

## CHAPTER I.

## CULTURES FROM MAMMALS, BIRDS AND FISHES.

THE following observations are extracted verbatim or in summary from the record of the Bacteriological Log :—

WEDDELL SEAL (*Leptonychotes weddelli*).

11-3-12. *Throat and Nose.*

Cultures from the throat and nose of a Weddell seal were made, immediately after the animal had been shot, on *serum agar* and *nutrient agar slopes*.

After four days at 37° C., there were well-marked colonies, fusing into a pale white smear, having a faint yellow tint over the surface. The two types were :—  
(a) Small, pale, circular colonies, and (b) larger, white colonies, quickly losing their circular form during growth and fusing to form an irregular, filmy, pale smear.

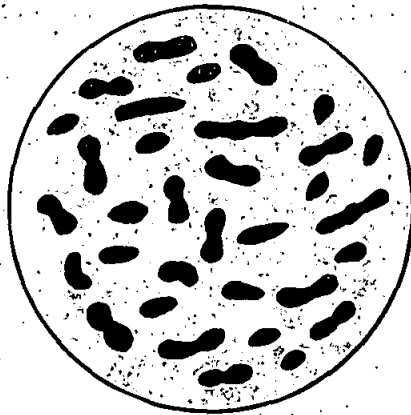
Smears stained by Gram's method showed :—

- (a) Streptococcal chains, Gram-positive.
- (b) A Gram-negative, sporing bacillus having short coccoid forms, and others constricted and dividing so as to resemble diplococci.

Subcultures of (a) and (b) were made and obtained each in pure culture.

- (b) 1. A *serum agar slope*, after 24 hours, showed pale, circular colonies growing rapidly and fusing into a filmy white smear.
- 2. On a *nutrient agar slope* there was a white growth spreading slowly.
- 3. A *gelatine stab* after a few days had a line of minute, white dots along the course of the stab; no liquefaction of the medium.

(b) 1, 2 and 3. Small, stout, Gram-negative bacilli, some with rounded ends, arranged singly and in groups of two; many, coccoid forms and pairs resembling diplococci.



Cultures of (b) examined after six days showed involuted forms of the bacillus; club-like bacilli and straight rods (short and long), many of which were spore-bearing.

After two weeks the culture ceased to grow; it was a filmy smear, pale yellow in tint. Involution forms of the bacilli were more marked, some being stout and curved, and many bulbous and bearing refractile spores.

Cultures made simultaneously from the nose and throat of a crab-eater seal had the same character:—

CRAB-EATER SEAL (*Lobodon carcinophagus*).

11-3-12. *Throat and Nose.*

On *serum agar* and *agar slopes*, after four days at 37° C., there were well-marked colonies fusing into a pale white smear having a faint yellowish tint. They were of two types:—(a) Small, pale, circular colonies, and (b) larger white colonies, quickly losing their circular form during growth, and fusing to form an irregular, filmy, pale smear.

(a) Gram-positive streptococci.

(b) A Gram-negative, sporing bacillus, having short, coccoid forms, and others constricted and dividing so as to resemble diplococci. After almost a week of growth, the bacilli were long, involuted, and spore-bearing.

*Weddell Seal—Wounds.*

It is a fact of some interest that most of the Weddell seals which managed to find a landing-place on the blizzard-swept shores of Cape Denison were covered with wounds, which had been recently received and were slowly healing, and with many old scars. The wounds varied from simple incisions to deep, ragged and extensive lacerations. Occasionally there was wide-spread destruction of tissue affecting the whole thickness of the layer of blubber, and invading the muscle. It is surmised that the Weddell seals, which live for a great part of the year on the floe-ice adjoining the foreshores or on the land itself, are subjected to the attacks of sea-leopards and killer whales, which abound throughout the zone of pack-ice. On one occasion two members of the Expedition actually observed a sea-leopard feeding off the carcase of a seal floating on the surface of the water. Certain it is that the majority of the land seals which came ashore by swimming across the open water against the continuous blizzard during the spring or autumn of 1912 and 1913—there were very few during the winter—had run the gauntlet of many attacks (Plate 14). One particular seal was so badly wounded with multiple tears and severe lacerations, and had lost so much blood, that it had just sufficient strength to land on the low, flat harbour-ice. This seal was observed for several days, during which all the wounds commenced to suppurate. Then the animal lapsed into a comatose state from the resulting septicæmia, and was eventually shot.

It may seem a fact contrary to the usual presuppositions about Antarctica that on every occasion in which these wounds were bacteriologically examined they were found to contain abundant pyogenic and other bacteria. Though the temperature of the surrounding air may be as low as 30° F., with blizzard winds up to and beyond one hundred miles per hour in velocity, multiplication of bacteria goes on in the warm interstices of the wounds, and it is possible to view every stage of disintegration from

slow, degenerative sloughing down to actual pus formation. And round a seal with many wounds one may often note small heaps of frozen pus and sanious exudation lying on the foot-ice.

Infection and suppuration are undoubtedly hastened because of the thick, protective layer (three to five inches) of blubber, with a relatively poor blood supply, which intervenes between the hairy skin and the muscle. Then, too, seals often lie for days on one patch of ice, which becomes covered with their own and other seals' excreta. So that it is not surprising to find *Bacillus coli communis* adding itself to an already mixed infection.

In eight cultures on *nutrient*, *glucose*, and *serum agar* which were made in Adelie Land from wounds of a Weddell seal on December 27th, 1913, and delivered for examination to Dr. Cleland, in Sydney, on March 3rd, 1914, the following bacteria were found:—Staphylococci, streptococci, a Gram-negative mycelium, Gram-positive yeast-like bodies and Gram-negative bacilli.

The following notes were made in the Bacteriological Log on March 9th, 1912:—

A wound about five inches long, one inch deep and half an inch wide, with a loose, sloughing centre was observed on the body of a Weddell seal lying on the foreshores. There was abundant exudation which had dropped in several places on to the ice to form yellow, frozen piles. The slough could not be secured, but some of the exudation was carried back to the hut for the purpose of making cultures. Smears were made from the suppurating wound of a young seal calf and stained with Jenner's.

- There were many degenerated leucocytes, very granular and crenated; apparently polymorpho-nuclear. Bacteria were very numerous; cocci of varying size were present as well as cocco-bacilli, grouped in pairs, and long, thick, bacillary rods. (Plate 13, figs. 15 and 16.)

Two cultures were made on *serum agar* from the pus of the adult seal's wound.

12-3-12. After 24 hours at 37° C., a fine, smear-like, somewhat opaque growth has appeared, and in 72 hours there was a well-defined, waxy growth with a dull-white sheen, along the line of the stroke. The bacteria present are streptococcal chains, staphylococcal bunches, cocco-bacilli arranged in pairs, and many curved and straight bacillary rods.

16-3-12. Subcultures, eight days later, show circular, white colonies (like *Staphylococcus albus*), a translucent smear (cocco-bacilli, arranged in pairs, mixed with short bacilli and longer bacillary rods); and a fine growth (streptococcal chains).

29-3-12. One culture from a seal's wound appears as a white, dry growth on *serum agar*; a pure culture of short, coccoid bacilli, many constricted so as to appear like diplococci.

Another culture in the form of a pale white, firm smear contains abundant streptococcal chains and cocci.

A third culture (stained with carbol fuchsin) has straight, thick, bacillary rods.



## WEDDELL SEAL—RECTUM.

1-3-12. A stab culture in *glucose agar* was made from the rectum of a Weddell seal. It was about ten days before a fine, branching growth appeared along the site of the stab. In several places there were small bubbles of gas in the medium.

Smears show long and short bacillary rods, some with deeply stained bipolar bodies; many were like *Bacillus coli communis* in appearance.

19-3-12. Anaerobic and aerobic cultures were made from the intestine of a Weddell seal on *nutrient*, *serum* and *glucose agar*.

A smear made from the large intestine, stained with carbol fuchsin, has :—

- (a) Many slender, straight, bacillary rods;
- (b) Short, thicker rods, some spore-bearing;
- (c) Long bacillary rods in chains; and
- (d) Cocco-bacilli and cocci.

25-3-12. *Ileum*. A *serum agar* culture shows fine, discrete, rather opaque colonies, examined after six days at 37° C. In a smear, stained with carbol fuchsin, there are long and short bacillary rods, coliform in appearance.

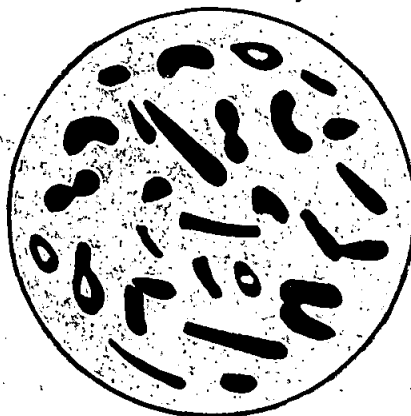
*Rectum. Glucose agar stab.* The medium has been broken up by the formation of gas bubbles; along the course of the stab there is a fine, greyish growth and numerous, minute, gas bubbles pushing out into the medium with the lateral processes of the growth.

A smear shows long, short, straight and curved bacilli, some spore-bearing.

26-3-12. *Sigmoid flexure. Gelatine stab.* The medium is full of gas bubbles as in the previous culture; a fine growth is observed pushing out in lateral offshoots into the medium; some liquefaction.

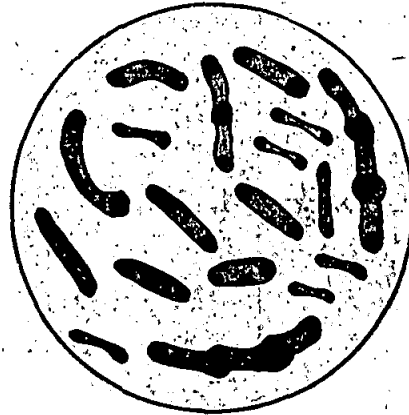
There are long and short, stout and slender bacillary rods.

27-3-12. *Rectum.* A *serum agar slope* has numerous greyish, circular growths which show a diversity of bacilli; slender, thick, short and long, straight and curved forms; a few cocco-bacilli; many spores and spore-bearing bacilli.



28-3-12. *Rectum*. An anærobic culture (*agar slope*) shows a filmy, grey smear along the course of the stroke. A few short bacilli very similar to *Bacillus coli communis*.

29-3-12. *Rectum*. The *glucose agar stab* (described on 25-3-12) has large, stout bacilli, many of which are curved. Bipolar staining is very noticeable in some bacilli; in others the deeply stained portions are eccentrically placed. There are also large, involuted forms containing as many as four dark granular bodies.



30-3-12. *Sigmoid flexure*. A subculture on *agar* made from the gelatine stab (26-3-12) has a single, circular, colourless colony, after 36 hours at 37° C. Long and short bacillary rods, some spore-bearing.

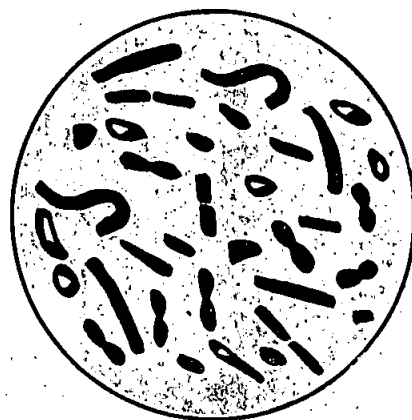
#### FROZEN FÆCES.

Frozen fæces of seals and penguins could be seen along the icy foreshores in the summer and autumn. During the winter the heavy falls of snow accompanying the blizzards obliterated all traces of excreta. The liquid fæces of the penguins are either pink—possibly from the large number of *Euphausia* (pink crustaceans) they consume—or dirty green from biliverdin pigment. The excreta of the Weddell seal are either blackish or chocolate-coloured.

30-3-12. One chocolate-coloured lump which had been, from observation, *in situ* for six weeks, was chipped off from the foot-ice and taken back to the Hut. An *agar slope* was inoculated, and after 48 hours at 37° C. is covered with a rapidly growing, greyish smear. Numerous short, stout bacilli and longer, thick forms; many are typically coliform in appearance.

16-10-12. *Weddell Seal. Rectum*. Two cultures (*agar slopes*), inoculated from the rectum, show greyish, circular growths which appear on smears as short and

longer, stout, somewhat coccoid bacilli (in length  $1\mu$  to  $3.6\mu$ ); some being spore-bearing; a few elongated and curved forms.



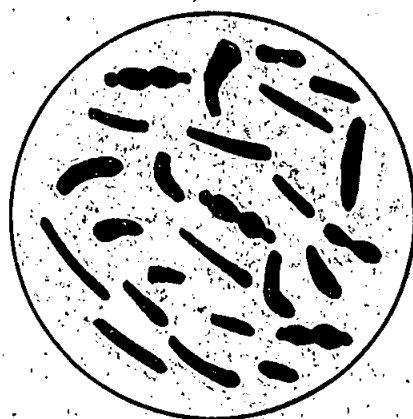
SEA-LEOPARD. (*Stenorhynchus leptonyx*.)

3-11-13. A smear from the large intestine, made on a glass slide and stained with carbol fuchsin, demonstrated the presence of many rod-like bacilli of various sizes, similar to those found in the intestine of the Weddell seal. No culture tubes were available.

ADELIE PENGUIN. (*Pygoscelis adeliae*.)

(1) 4-3-12. *Rectum*. An *agar slope*, after three days at  $37^{\circ}$  C., had along the course of the stroke a fine, glistening, greyish smear which died out and dried, after six days. In smears there were short, stout bacilli showing bipolar staining.

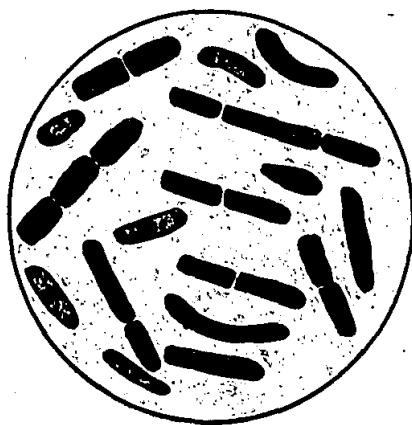
7-3-12. A *glucose agar shake* culture was, after a few day, broken up extensively by the formation of gas bubbles in the medium. A few greyish, elliptical growths could be discerned. Stained smears contained long and short, stout, and, in some cases, curved bacillary rods.



13-3-12. An anærobic culture on an *agar slope* (using pyrogallie acid and potassium hydrate), is ridged along the course of two strokes, but no organisms can be found.

(2) 1-4-12. *Rectum*.—A smear, stained with carbol fuchsin, shows long and short, stout bacilli and a few slender forms.

*Lower Intestine*.—Numerous bacilli, long, short and stout; some chained, some with polar staining, or with a deeply stained area near the middle of the bacterium.



3-4-12. *Intestine*.—A serum agar slope, after 48 hours, shows a milk-white growth terminating below in a greyish smear. The white growth appears as cocci and staphylococcal bunches; the lower part of the growth as bacilli of a coliform type.

*Intestine*.—A pale, greyish smear on an agar slope after 48 hours. Slender block-like bacilli (see sketch), some arranged in short chains and dense packets, with organisms of the coliform type, as in the previous culture. Bipolar staining was marked in some cases.



*Intestine*.—Glucose agar shake. The medium is disrupted by gas bubbles; no definite growths can be made out, although smears from cloudy areas contain a few moderately long, straight, stout bacilli.

8-4-12. *Lower Intestine*.—An anærobic culture on an agar slope is visible after 9 days at 37° C., as a pale, glistening, somewhat gelatinous, warty growth, with some

corrugation and pitting. The bacilli are represented as short and long, slender rods; the longer ( $15\mu$  to  $20\mu$ ) being much attenuated, curved and polymorphic in character.



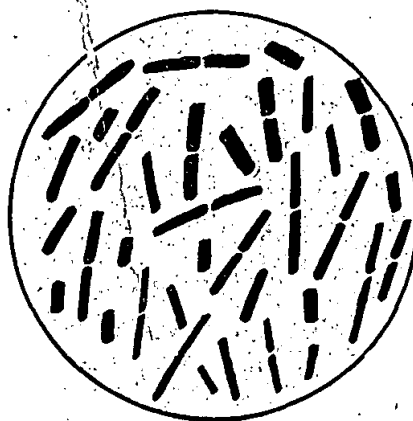
Unfortunately this culture was lost.

10-5-12. *Frozen Excresc.*—A specimen was secured from the snow adjoining a rookery, six weeks after the birds had gone to sea.

After four days at  $37^{\circ}\text{C}$ , an agar slope shows a brownish, smeary growth, partly overgrown with waxy, white colonies.

Cocci in staphylococcal bunches and bacilli very similar to *Bacillus coli communis*.

14-10-12. A scraping from the lower intestine (stained with Leischmann) has epithelial cells, red blood corpuscles, leucocytes and numerous bacilli rod-shaped and block-like ( $2\mu$  to  $3.5\mu$  in length) in character.



19-10-12. *Lower Intestine*.—An anærobic agar slope culture, after five days at  $37^{\circ}\text{C}$ , has grown two circular, dull-white colonies at the upper part of the slope; there is (?) contamination below by a growth somewhat similar to *Bacillus subtilis*. A few cocci and cocco-bacilli observed.

SOUTHERN SKUA GULL (*Megalestris antarctica*).

15-3-12. *Small Intestine*.—A cover-glass preparation (stained with carbol fuchsin) shows a few cocci and cocco-bacilli. Bacteria are very scarce over the whole field.

*Rectum*.—Many bacteria, coliform in appearance, associated with cocci and cocco-bacilli; a few long, curved forms.

18-3-12. Two types of colonies are visible on a *serum agar slope*, after three days at 37° C. :—

- (a) A pale, greyish growth of irregular form;
- (b) Discrete, somewhat opaque, small circular colonies.
  - (a) Short bacillary rods coliform in type.
  - (b) Long and short, stout bacilli; some are more slender and curved; some are sporing, and others are in short chains.

A subculture was made from (b).

*Small Intestine*.—The *serum agar slope* (18-3-12) is covered with a generalised brownish smear. Short rods, associated with a few longer forms; a few cocco-bacilli.

*Small Intestine*.—Along the course of a stroke on *nutrient agar* there is a linear, pale, greyish smear. Bacilli very similar to *Bacillus coli communis*, together with a longer sporing bacillus.

19-3-12. *Rectum*.—The subculture from (b) has somewhat opaque, small, circular colonies. Numerous thick bacilli, of varying length, sporing.

*Rectum*.—A stab in *glucose agar* shows after a few days an air-bubble along the course of the needle. Another larger bubble invades the medium. There is a fine greyish growth. Bacilli, coliform in appearance.

*Rectum*.—A *glucose agar shake* culture has become infected with mould.

27-3-12. The subculture of a whitish colony, made on 19-3-12, is apparent on an *agar slope*, after eight days' incubation at 37° C., as a white, waxy growth. Small, short, slender bacilli, sporing.

*Rectum*.—The subculture on *agar* of a fine, pale colony appears as a diffuse pale growth; cocco-bacilli similar to forms previously seen.

SNOW PETREL (*Pagodroma nivea*).

29-4-12. In smears (stained with carbol fuchsin) from the upper and lower intestine (near the rectum) the micro-organisms were very sparse. A few cocco-bacilli were observed, after looking over several large fields.

Cultures on *serum* and *nutrient agar* from the intestine and rectum produced no growth. Along the course of a stab in *gelatine* a faint, pale yellow fringe was observed, but no bacteria could be stained.

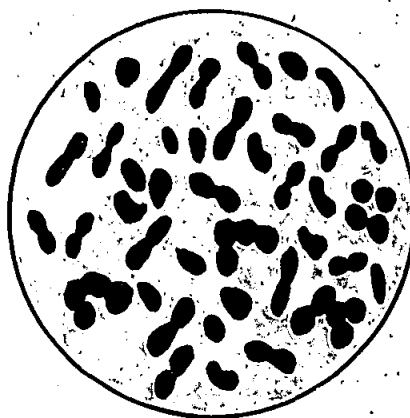
11-5-12. *Lower Intestine*.—After 14 days' at 37° C., a *glucose agar shake* culture contains a few, minute, greyish elliptical growths (viewed with a lens); no formation of gas.

Smears demonstrate numerous micrococci and cocco-bacilli.

*Small Intestine*.—A fine, greyish growth appeared on a *serum agar slope* after 10 to 14 days at 37° C. Some large cocci and cocco-bacilli similar to those seen in the previous culture.

*Lower Intestine*.—A *serum agar* culture, after 10 to 14 days, has a brownish smear along its sloped surface. Long and short bacilli, some chained; a few cocco-bacilli.

An *agar slope* subculture, made from the preceding, produced, after 48 hours at 37° C., a rapidly growing, dull-white smear with a few outlying circular colonies. Long and short, thick and slender bacilli (none coliform in appearance); large cocci and cocco-bacilli as below:—



#### ANTARCTIC PETREL (*Thalassœca antarctica*).

29-4-12. A smear (stained with carbol fuchsin) from the rectum shows a very few, straight, slender bacilli (5 $\mu$  to 6 $\mu$  in length), some cocci and (?) cocco-bacilli, epithelial cells and debris.

In a smear from the lower intestine there are slender, straight bacilli similar to those seen in the rectum, but more numerous.

No bacteria can be seen in the smear from the upper intestine.

*Lower Intestine*.—No growth was obtained on *serum agar*.

*Rectum*.—No growth was obtained on *serum agar*.

*Lower Intestine*.—No growth along a stab in *gelatine*, incubated at 15° to 18° C. No liquefaction of the medium or obvious gas-formation. The *gelatine* was very brittle and split in every direction.

11-5-12. *Lower Intestine*.—In a *glucose agar shake* culture there are a few straw-coloured, lenticular growths, after 10 to 14 days at 37° C. Some cocci and diplococci.

27-9-12. An Antarctic petrel was examined 48 hours after death. Smears (stained with carbol fuchsin) from the stomach, jejunum, ileum, lower intestine and rectum were made. A few very slender, short bacilli were seen; some cocco-bacilli (?) and cocci. There was a marked absence of bacteria in all the smears, and one had to traverse many fields before picking one up.

Culture tubes were inoculated from the small intestine, lower intestine and rectum.

1. *Small Intestine*.—Small, white, papilla-like colonies grew on a *serum agar slope*. Cocci and diplococci in masses; large cocci (twice the diameter of the first-named); cocco-bacilli, and a very few, slender, bacillary rods.

2. *Large Intestine*.—Small, circular, discrete, pale colonies were visible on an *agar slope*. Cocci and diplococci, separate and in bunches.

3. *Rectum*.—There were similar colonies on *agar* to those seen in the previous culture. Cocci and diplococci, separate and in bunches.

4. *Small Intestine*.—A pale, fine growth appeared along the course of the stroke on *agar*. Large cocci similar to those found in (1.)

Subcultures were made from (1) and (2).

(1) *Glucose agar slope*.—(a) A pale, liquid smear, and (b) a few circular, white colonies.

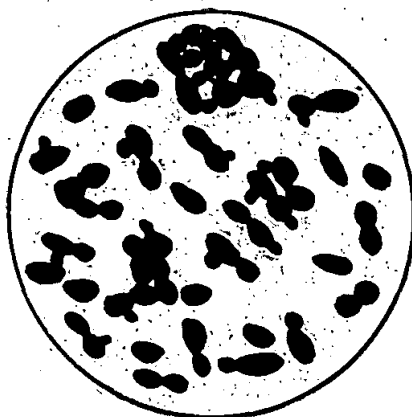
(a) Yeast torulæ.

(b) Small cocci in masses.

(2) The *agar slope* has (a) circular, glistening white colonies growing rapidly, and (b) a pale smear.

(a) Bacillary rods, block-like,  $1.4\mu$  to  $2.8\mu$  in length; and

(b) Yeast-like organisms.



Several of the subcultures afterwards became infected with mould.



COD FISH (*Nototheniae*).

It appears, from scientific reports which have already been published, that *Notothenia coriiceps* is the species most commonly found in and around the boat harbour at Cape Denison, Commonwealth Bay. The temperature of these fish corresponds almost exactly with that of the sea-water (usually about 29° F.).

They and another similar species, *Trematomus bernacchii*, seem to be the only fish inhabiting the shallower waters close to the land; in fact they live amidst the kelp weed, and it is here that they may be caught most readily by hand-lines or traps. In appearance they are very similar to the small "rock-cod"—and present the same variety in colouration—which thrive amongst the inshore rocks and seaweed anywhere along the coast of Australia.

It is probably on account of their more or less secluded existence amongst the serpentine masses of kelp that these small fish escape the depredations of seals, sea-leopards, and sea-elephants. In fact, when they are very young, they are liable to be eaten by penguins, which for at least six months of the year infest in their millions the shores of rocky outcrops and islands fringing sparsely the ice-bound coastline of Antarctica. So it may be understood that fish are relatively scarce in the shallow coastal zone, and the same rule holds good in the case of Macquarie Island, situated in Sub-Antarctica.

Two cultures were made from *Notothenia coriiceps* :—

10-5-12. *Lower Intestine*.—(*Notothenia coriiceps*). A serum agar slope, after 24 hours at 15° to 18° C., shows a few pale, circular colonies, each at the bottom of a pit of liquefaction in the medium. Slender bacilli, long and short.

*Rectum*.—(*Notothenia coriiceps*). After 48 hours at 37° C. an agar slope has a few small, circular, pale, slowly growing colonies. Large cocci in staphylococcal masses and some cocco-bacilli.

## SMEARS FROM MAMMALS, BIRDS, AND FISH.

A large number of slides were prepared, at various times during 1912 and 1913, from smears of intestinal contents. These were mostly stained with carbol fuchsin and packed away in boxes. The slides were not finally examined until three years after their arrival in Australia, owing to unavoidable circumstances. Meanwhile they had been kept in a dark cupboard and were in a good state of preservation when viewed under a microscope. The particular smears described below were, first of all, decolourised in acid alcohol, and then re-stained by Gram's method.

WEDDELL SEAL (*Leptonychotes weddelli*).

*Stomach*.—Bacteria are not numerous.

- (1) Small, oval, Gram-negative bacilli ( $\cdot 5\mu$  in length,  $\cdot 2\mu$  in width), occurring typically in twos and like diplococci in appearance.
- (2) Gram-positive micrococci ( $\cdot 2\mu$  in diameter) were in greatest numbers.

- (3) Some slender, Gram-negative bacilli (about  $2\mu$  in length).

*Small Intestine.*—Bacteria are plentiful.

- (1) Oval, Gram-negative bacilli ( $2\mu$  in length,  $1\mu$  in width), showing more elongated and definite bacillary forms and occurring in twos, adhering together; the smaller groups closely resemble diplococci.
- (2) Many Gram-positive cocci ( $.4\mu$  to  $.5\mu$  in diameter).
- (3) Slender, Gram-negative bacilli of varying length; often in twos or short chains.
- (4) Gram-negative, coliform bacilli are represented.
- (5) Gram-positive micrococci ( $.2\mu$  in diameter).

*Rectum.*—Bacteria are very numerous.

- (1) Stout, Gram-negative, sporing bacilli of variable size ( $4\mu$  in average length,  $1.2\mu$  in width) are most in evidence. Their arrangement is commonly in twos, end to end.
- (2) Slender, Gram-negative bacilli ( $3.4\mu$  long,  $.3\mu$  wide) occur singly, in twos or in short chains. Some bacilli have small nodules deeply stained, or clear spaces in their protoplasm.
- (3) Gram-negative, coliform bacilli.
- (4) Gram-positive cocci ( $.3\mu$  in diameter) and diplococci.

#### SEA-LEOPARD (*Stenorhynchus leptonyx*).

*Stomach.*—Bacteria are sparse in numbers.

- (1) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.
- (2) Gram-positive, short, slender bacilli ( $.6\mu$  long and  $.1\mu$  wide).

*Small Intestine.*—Bacteria are in moderate numbers.

- (1) Slender, gram-positive rods ( $1.8\mu$  in length,  $.2\mu$  in width).
- (2) Stout, Gram-negative bacilli ( $3.4\mu$  in average length,  $.6\mu$  in width) which are variable in length.
- (3) Slender, Gram-negative bacilli, with short and long forms.
- (4) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci.

*Caecum.*—Bacteria are very plentiful.

- (1) Stout, Gram-negative, sporing bacilli ( $4.1\mu$  in average length,  $1.3\mu$  in width), most often arranged in twos, end to end.
- (2) Very slender ( $.1\mu$  in width), Gram-negative rods ( $2\mu$  in length), occurring singly or in chains of three or four.
- (3) Gram-negative, coliform bacilli.
- (4) Gram-positive cocci ( $.3\mu$  in diameter) and diplococci.

ANTARCTIC PETREL (*Thalassoeca antarctica*).

*Stomach*.—Many bacteria are seen, chiefly (2).

- (1) Slender, Gram-positive bacilli ( $3.4\mu$  in length,  $.3\mu$  in width).
- (2) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci.

*Small Intestine*.—Bacteria are in fair numbers.

- (1) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter).
- (2) Very slender, short, Gram-positive bacilli ( $1.5\mu$  in length,  $.2\mu$  in width); some elongated forms.
- (3) Gram-positive, bean-shaped cocci in pairs ( $.5\mu$  in longest diameter).
- (4) Short, Gram-negative cocco-bacilli ( $.5\mu$  in length).

*Rectum*.—Bacteria are in moderate numbers.

- (1) Many Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.
- (2) Gram-negative, somewhat coliform bacilli.
- (3) Gram-negative, stout rods ( $3.4\mu$  in average length,  $.9\mu$  in width).

SNOW PETREL (*Pagodroma nivea*).

*Stomach*.—Bacteria are fairly numerous.

- (1) Large, Gram-positive diplococci ( $1.1\mu$  in diameter), are most obvious.
- (2) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.
- (3) A few short, slender, Gram-positive rods.

*Small Intestine*.—Gram-positive micrococci and diplococci are plentiful; no bacilli seen.

*Rectum*.—Bacteria are numerous.

- (1) Gram-positive diplococci ( $1.1\mu$  or more in diameter) are most plentiful.
- (2) Slender, Gram-positive rods (from  $2.5\mu$  to  $5\mu$  in length,  $.2\mu$  in width).
- (3) Gram-positive bacilli ( $2.5\mu$  in average length,  $.6\mu$  in thickness), arranged singly or in chains of three or more.
- (4) Thick, Gram-negative, sporing bacilli ( $2.5\mu$  long,  $.8\mu$  wide), somewhat variable in length.
- (5) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.

GIANT PETREL (*Ossifraga gigantea*).

*Stomach*.—Bacteria are in fair numbers.

- (1) Short, slightly curved, Gram-negative bacilli.
- (2) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci are in greater number.

*Small Intestine*.—Bacteria are in moderate numbers.

- (1) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci are in greater number, often occurring in masses.
- (2) Some short, Gram-negative bacilli; a few curved.

*Rectum*.—Bacteria are very plentiful. (Plate 13, fig. 13).

- (1) Stout, Gram-negative bacilli ( $3.4\mu$  to  $8.5\mu$  in length,  $.8\mu$  in average width), arranged singly or in short chains of two or three bacilli; single, spore-bearing forms.
- (2) Gram-negative bacilli similar to *Bacillus coli communis*.
- (3) Gram-positive cocci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci.

WILSON PETREL (*Oceanites oceanicus*).

*Stomach*.—Bacteria are very sparse. A few Gram-negative cocco-bacilli ( $1\mu$  in length, or smaller).

*Small Intestine*.—No bacteria are seen.

*Rectum*.—Bacteria are few in numbers.

- (1) Very slender, Gram-negative rods, most often in short or long chains ( $3.4\mu$  in average length,  $.1\mu$  in width).
- (2) Gram-positive cocci ( $.2\mu$  in diameter) and diplococci.

PRION (*A new species, very similar to Prion banksii*).

*Stomach*.—No bacteria are visible.

*Rectum*.—Bacteria are very sparse.

- (1) A few, very slender, Gram-negative rods ( $1.7\mu$  in length,  $.1\mu$  in width).
- (2) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.

EMPEROR PENGUIN (*Aptenodytes forsteri*).

*Stomach*.—Bacteria are in moderate numbers.

- (1) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci are in greatest number.
- (2) Thick, Gram-negative bacilli ( $3.4\mu$  long,  $.9\mu$  wide).
- (3) Gram-negative cocco-bacilli ( $1\mu$  in length,  $.8\mu$  in width).

*Small Intestine*.—Bacteria are fairly numerous.

- (1) Gram-negative bacilli ( $3.5\mu$  in average length,  $.9\mu$  in width) which vary greatly in size; they are arranged singly or in twos. Some elongated forms reach  $14\mu$ .
- (2) Many Gram-negative bacilli are like *Bacillus coli communis* in appearance.
- (3) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci.

*Rectum*.—Bacteria are very numerous.

- (1) Thick, Gram-negative bacilli ( $3.4\mu$  in average length,  $1.1\mu$  in width) having elongated and curved forms.
- (2) Gram-negative bacilli, very similar to *Bacillus coli communis*.
- (3) In greatest number are masses of Gram-positive cocci ( $.2\mu$  to  $.3\mu$  in diameter).

ADELIE PENGUIN (*Pygoscelis adeliae*).

*Stomach*.—Bacteria are very few.

- (1) Single, Gram-positive bacilli ( $1.7\mu$  in length,  $.4\mu$  in width).
- (2) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci.

*Duodenum*.—Bacteria are sparse.

- (1) Gram-positive micrococci ( $.2\mu$  in diameter).
- (2) Slender, Gram-positive bacilli ( $2\mu$  in length,  $.3\mu$  in width).

*Small Intestine*.—Masses of Gram-positive cocci ( $.2\mu$  to  $.3\mu$  in diameter) are numerous; there are cocci in short chains; no bacilli seen.

*Rectum*.—Bacteria are very plentiful.

- (1) Numerous spore-bearing, Gram-positive bacilli ( $3.4\mu$  in average length,  $.9\mu$  in width) occurring singly or in twos, end to end, at an obtuse angle.
- (2) Gram-negative bacilli very similar to *Bacillus coli communis*.
- (3) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci.

SKUA GULL (*Megalestris antarctica*).

*Stomach*.—Some Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.

*Small Intestine*.—Gram-positive micrococci ( $.2\mu$  in diameter) seen; no bacilli.

*Rectum*.—Bacteria are very numerous.

- (1) Gram-negative bacilli, very similar to *Bacillus coli communis*.
- (2) Stout, Gram-negative bacilli ( $3.5\mu$  in average length), bearing in some cases refractile spores; often in pairs, end to end; some elongated forms.
- (3) Slender, Gram-positive rods ( $2.2\mu$  in length,  $.3\mu$  in width).

No cocci seen in the smear.

COD FISH (*Notothenia coriiceps*).

*Stomach*.—Bacteria are few in numbers.

- (1) Slender, Gram-negative bacilli ( $1.7\mu$  in length,  $.2\mu$  to  $.3\mu$  in width), some a little curved; elongated, curved forms occasionally seen.
- (2) Gram-positive micrococci ( $.2\mu$  in diameter) and diplococci.
- (3) Gram-negative cocco-bacilli ( $1\mu$  long,  $.5\mu$  wide).

*Intestine*.—Bacteria are fairly numerous.

- (1) Slender, Gram-negative bacilli of varying length ( $3.4\mu$  in average length,  $.2\mu$  in width); mostly single, otherwise in pairs.
- (2) Gram-positive micrococci ( $.2\mu$  to  $.3\mu$  in diameter) and diplococci in greatest number.
- (3) A few small, Gram-negative cocco-bacilli.

## CHAPTER II.

## ANTARCTIC BACTERIOLOGICAL INVESTIGATIONS.\*

## CULTURES FROM ANIMALS.

CULTURES from a number of mammals and birds killed in Antarctica were received. In a number of instances, as for example in the case of the Ross Seal, no bacteria had grown on the culture media. In the majority of instances, however, growth of a coliform type was present, and these cultures were fully investigated to determine the type of bacillus present. In a few cases sporing bacilli were met with, and in one case where cultures were made from the wound of a seal, streptococci and staphylococci were found.

Most of the cultures were made in these cases from the rectum of the killed animal, which would account for the preponderance of coliform bacilli. The cultures were mostly on agar slopes, but gelatine and serum slopes and glucose agar stab-cultures were made in a number of cases.

CRAB-EATER SEAL (*Lobodon carcinophagus*).

Six cultures from this animal were received.

- 8-1-14. Agar stab.—No growth.
- 8-1-14. Agar slope.—Coliform growth.
- 16-1-14. Agar slope.—No growth.
- 16-1-14. Gelatine.—No growth.
- 17-2-14. Gelatine.—Growth; liquefaction.
- 17-2-14. Agar slope.—Coliform growth.

Subcultures were made from all on agar slopes. No growth occurred on three; and as the original cultures had shown no growth, the former were discarded. The remaining three showed a coliform growth.

Emulsions were made from the subcultures, and one loopful of each was plated on agar. Six colonies were then picked off from the plates on to agar slopes. Two of the cultures showed coliform organisms only, the third showed coliform bacilli and a Gram-positive, sporing bacillus.

The coliform organisms were tested on the "first five sugars."\* The sporing bacillus was plated and also put on to gelatine and potato.

On agar plates, its colonies were whitish, large and irregular, flat, not raised in the centre, edges irregular, filamentous, giving them a fluffy appearance.

On agar slopes a dense, white, film-like growth was produced.

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\* The following report, compiled by Drs. J. B. Cleland, E. W. Ferguson and K. Smith, is a summary of part of the work done on various specimens submitted to the Bureau of Microbiology, Sydney.

† Vide Table.

On gelatine, liquefaction occurred in 24 hours. After four days, liquefaction extended to the depth of the stab, and growth was in the form of a flocculent deposit at the bottom of the liquid.

On potato. After four days, growth is slightly moist, film-like, slightly wrinkled, greyish-yellow in colour, but pale around the edges.

WEDDELL SEAL (*Leptonychotes weddelli*).

Five cultures were submitted from this animal.

Two glucose agar stab-cultures from the rectum, one dated 16th December, 1913, and another undated, showed no growth. These were replated but with negative results.

The other three cultures, on sloped agar, showed a growth of Gram-negative bacilli of a coliform type.

One tube was labelled, "Seal Rectum, 2nd Jan., 1914." The other two were subcultures, and were dated "2nd Feb." and "17th Feb.," respectively.

These cultures were subcultured, and emulsions were made from the growth. A loopful of emulsion from each was plated on agar, and six colonies were picked off on to agar slopes and inoculated into the "first five sugars."

Glucose agar stabs were made from the original stab-cultures submitted. Apparently no growth took place. On staining the originals, short and long Gram-negative bacilli were present. The same organisms were found to be present in the subcultures on making smears.

WOUND OF WEDDELL SEAL (*Leptonychotes weddelli*).

Cultures were forwarded which had been made from the wound of a Weddell seal. Six original cultures were made; four on sloped agar, one stab in glucose agar, and one on serum. These were dated, "27th Dec., 1913." In addition, two subcultures on sloped agar and dated respectively, "26th Jan., 1914," and "17th Feb., 1914," were sent.

Cultures dated.					No. in Laboratory List.		
27-12-13	Agar slope	...	...	...	...	...	2
"	"	...	...	...	...	...	3
"	"	...	...	...	...	...	4
"	"	...	...	...	...	...	6
"	Serum slope	...	...	...	...	...	5
"	Glucose agar stab	...	...	...	...	...	8
26-1-14	Agar slope subculture	...	...	...	...	...	1
17-2-14	"	...	...	...	...	...	7

From these eight cultures, twelve subcultures were made on agar, on the 24th February, 1914.

Subcultures.

- No. 1. Gram-positive cocci in chains.
- 2.               "               (staphylococci).
- 2b.             "             in chains.
- 3.             "             (staphylococci).
- 3b.            "            (staphylococci).
- 4.             "             (streptococci).
- 4b. Gram-negative mycelium, Gram-positive yeast-like bodies.
- 5. Gram-positive cocci (staphylococci).
- 6.             "             "
- 6b. (?) No growth.
- 7. Gram-positive cocci (streptococci).
- 8. Gram-negative, (?) mycelium or bacilli.

Further subcultures were made on 24th March, 1914. Nos. 1, 2b, 4, 7, showed fine colonies of Gram-positive cocci (? streptococci). From these, tubes of bouillon were inoculated in which a fine flocculent growth, gravitating to the bottom of the tube, was produced. On staining, long chains of streptococci were present.

These bouillon cultures were then plated, and separate colonies picked off and tested on the "sugars."

Subcultures Nos. 2, 3, 3b, 5, 6, showed a dense white growth like that of *Staphylococcus albus*.

Subculture No. 4b showed no evident growth, but on staining, a few lengths of mycelium were detected. Later the culture was overgrown with a mould.

Subculture No. 8 showed a doubtful, very fine growth. On staining, irregular Gram-negative bacilli, moderately long and thick, were found to be present.

The subcultures showing *Staphylococcus albus* were inoculated on to gelatine, and left at room temperature.

Nos. 3, 3b, 5, 6, showed a whitish growth down the needle track, spreading out on top. No liquefaction of the gelatine was produced in 24 hours; after four days slight liquefaction appeared at the top.

No. 2 produced liquefaction of the gelatine in 24 hours, forming a dense, white precipitate at the bottom of the liquid, and a fine growth above it; the liquid was turbid.

The streptococci were tested on the "sugars"; three colonies being picked off from each plate and inoculated into glucose, mannite, lactose, saccharose and raffinose, and also into litmus milk.

After 72 hours no change was observed in these media.



After 21 days, one culture from No. 4 and one from No. 7 showed no change; the other cultures all showed acid in glucose.

No change was produced in the milk.

In gelatine, liquefaction was observed in all the cultures.

#### WANDERING ALBATROSS (*Diomedea exulans*).

Three cultures on sloped agar, gelatine and glucose agar (stab), made 200 miles south of Tasmania, were submitted. The cultures were dated 24th February, 1914, and one (the agar slope culture) was labelled "rectum," the others not being so labelled, though presumably these cultures were also made from the rectum. On receipt, subcultures on sloped agar were made from the original cultures and showed Gram-negative bacilli. Emulsions were then made from the subcultures and plated on agar; one loopful of the emulsion being added to each plate. After incubation, each plate showed an extensive growth of confluent colonies of a coliform type. Six colonies were picked off from each plate and cultured on agar. From these cultures, which showed a pure growth of a Gram-negative, colon-like bacillus, the "first five sugars" (glucose, mannite, dulcitol, lactose and saccharose), were inoculated.

In all three cases the six subcultures showed the same reactions on the "sugars," giving "A. and G." after 72 hours' incubation. As the "sugars" reactions of all the coliform organisms isolated from the various animals were determined at the same time, it will be more convenient to discuss them together.

#### SKUA GULL (*Megalestris antarctica*).

Eight cultures from the skua gull were received, the original cultures having been taken from the rectum of the bird, and subcultures made at intervals. In the following list, the cultures are arranged in accordance with the dates on the tubes:—

16-12-13	Agar slope	...	...	...	Coliform growth.
„	Glucose agar stab	...	...	...	Growth.
6-1-14	Subculture (?) agar slope	...	...	...	Coliform growth.
14-1-14	Subculture (?)	...	...	...	Fine growth.
14-1-14	Agar slope	...	...	...	Coliform growth.
26-1-14	Subculture, agar slope	...	...	...	Coliform growth.
7-2-14	Subculture, gelatine	...	...	...	Growth.
17-2-14	Subculture, agar slope	...	...	...	Coliform growth.

Subcultures were made from all the cultures on agar slopes. In all, Gram-negative bacilli of a coliform type appeared. In the subculture from the gelatine, large Gram-positive bacilli were also present.

Emulsions were made from the agar subcultures and plated on agar, one loopful to each plate. Three colonies were picked off from each plate, subcultured on agar and transferred to the "first five sugars."

## SUGAR REACTIONS.

	MILK.			Glucose.	Mannite.	Dulcite.	Lactose.	Cane Sugar.	Maltose.	Dextrin.	Galactose.	Inulin.	Amygdalin.	Salicin.	Arabinose.	Raffinose.	Sorbitol.	Erythrite.	Inositol.	Adonite.	Remarks.
	1 Day.	3 Days.	15 Days.																		
Albatross ...	A	A	C	AG	AG	AG	AG	AG	AG	...	AG	...	...	AG	AG	AG	AG	...	...	...	2 cultures; 7 days' readings.
Skua Gull...	A	A	C	AG	AG	AG	AG	AG	AG	...	AG	...	...	...	AG	AG	AG	...	...	...	" "
Weddell Seal	A	C	C	AG	AG	AG	AG	AG	AG	...	AG	...	...	AG	AG	AG	AG	...	...	...	" "
Crab-eater Seal	2	A	A	C	AG	AG	AG	AG	AG	...	AG	...	...	AG	AG	AG	AG	...	...	...	1 culture; 7 days' readings.
"	5	A	A	C	AG	AG	AG	AG	AG	...	AG	...	...	...	AG	AG	AG	...	...	...	" "

Coliform organisms from the wandering albatross, Weddell seal, crab-eater seal and skua gull were tested on the "sugars."

The procedure adopted for the isolation of the coliform bacilli was the same in all cases. It consisted of subculturing from the original cultures, plating on agar an emulsion from the subcultures—one loopful to each plate—and picking off from the plate separate colonies on to agar slopes. From these agar slopes the organisms were inoculated on glucose, mannite, dulcite, lactose and saccharose.

In these five "sugars" all the cultures tested gave the same reactions; the formation of acid (A.) and gas (G.) after 72 hours' incubation. In some cases the 24 hours' reading showed no change in the dulcite, but this substance was in every case fermented in 72 hours. On glucose acid was frequently shown, but no gas. This result occurred also to some slight extent on saccharose, and was probably due to the excess formation of acid inhibiting the formation of gas. In some instances the colour of the medium was partially discharged; this occurring along with gas formation, and to a certain extent making the reaction acid. This discolouration was not confined to the "sugar," but occurred most frequently in media containing dulcite and saccharose.

The coliform organisms thus tested would fall according to their reactions into the *Friedlander-Neapolitanus* group. To determine whether more than one type belonging to this group were present, the organisms were further tested on the remaining "sugars." The results are shown on the accompanying table. In each case two subcultures derived from two separate original cultures were tested on the "sugars."

The results of all the tests were the same, with the exception of the reaction in salicin. Both cultures from the albatross and Weddell seal, and one from the crab-eater, gave "A. and G." on salicin, while both cultures from the skua gull and one from the crab-eater gave no change on this medium.

It is questionable if the difference on salicin alone is sufficient to differentiate the organisms into two types.

Probably if further cultures had been tested, both types would have been found present in each animal, and it is possible, though I do not think probable, other types of coliform organisms also.

Cultures from the following animals and birds gave negative results:—

#### ROSS SEAL.

Three agar slopes, a glucose agar stab and a gelatine stab were sent from this animal. No growth was apparent on the cultures, and subcultures showed no growth.

PRION (of a new species like *Prion banksii*).

16-12-13.	Rectum.	Agar slope.	No growth.
"	"	"	"
"	"	Gelatine.	"
26-12-13.	"	Serum.	"
"	"	Agar stab.	"
14-1-14.		Agar slope.	"
27-1-14.		Agar slope.	"

Subcultures were made from these, but showed no growth.

#### ANTARCTIC PETREL.

31-12-13.	Agar slope.	No growth.
22-2-14.	"	"

#### SILVER PETREL.

30-12-13.	Serum.	No growth.
"	Agar slope.	"
"	Gelatine.	"

#### SNOW PETREL.

23-12-13.	Agar slope.	Fine growth.
-12-13.	" stab.	No growth.
14-1-14.	" slope.	"
.....	Gelatine.	"

The culture showing (?) fine growth was subcultured, and again apparently a fine growth appeared. But on staining, no organisms were to be seen, and further subcultures showed no reappearance of growth.

## TERN.

16-1-14. Agar slope. No growth.

16-1-14. Agar stab. (?) Growth near top.

Two subcultures from the agar stab were made on agar slopes, but no growth appeared.

## PENGUINS.

17-12-13. Rectum. Agar slopes. No growth.

" " Gelatine. "

" " Agar stab. "

-12-13. Serum. One small colony.

14-1-14. " No growth.

The culture tube showing the single colony was put aside for further examination, but on re-examination the culture tube was found to have dried up and subcultures made after moistening the surface with a sterile brush showed no growth.

## HOLOTHURIAN.

22-12-13. Agar slope. (?) Slight growth. Smears showed nothing

31-12-13. " No growth.

COD (*Notothenia coriiceps* and *Chalinura ferrieri*).

Eight cultures from cods were received, all from the rectum. Several were labelled "Cod," and the others "Notothenia." The cultures were afterwards found to be from two species of fish.

Of the eight original cultures, four on examination showed no growth. The remaining four showed (?) growth or a fine growth. The first of these was taken in Adelie Land; the remaining three were recovered from the deep-sea trawl in latitude 64° 34' South, longitude 127° 17' East, at a depth of 1,700 fathoms; temperature -0.3° C.; thick ooze and rocks.

6-1-14. *Notothenia coriiceps*. Agar slope. Growth.

14-1-14. *Chalinura ferrieri*. Serum. Liquefied (?).

1,700 fathoms.

29-1-14. " " " (?) Growth.

17-2-14. " " " "

The cultures which showed no growth were dated "3-12-13, 20-1-14 and 21-1-14."

Subcultures were made from the four cultures apparently showing growth. Two of these showed no growth on subcultures. The remaining two showed (?) fine growth as follows :—

6-1-14.	(?) Fine growth.	Stained ...	Slender rods, Gram-negative; larger rods (? mycelium) Gram-negative.
29-1-14.	(?) Fine growth.	Stained ...	Slender rods, Gram-negative, sporing.

Further subcultures were made, but showed no growth, the organisms having apparently died.

#### CULTURES FROM PENGUIN GUANO.

A number of sealed test tubes were submitted containing guano and other material which had been gathered on the site of rookeries belonging to the Adelie penguin (*Pygoscelis adeliae*). The contents of each of these tubes were treated in the following fashion :—

A tube was opened and a small portion of its contents was emulsified in sterile tap water. From this emulsion two agar plates were inoculated in each case; the emulsion being thickly smeared over the surface. One plate was labelled "A" and the other "B."

From the same emulsion tubes of glucose broth covered with oil were inoculated, and also glucose agar slopes, these being grown under anaerobic conditions.

- I. Soil near penguin rookery containing guano, Cape Denison. 19-12-13.
- II. Penguin guano, Stillwell Island. 30-12-13.
- III. Penguin guano, Mackellar Islets. 19-12-13.
- IV. Penguin guano and soil from a rookery, Cape Denison. 19-12-13.
- V. Brine and a little penguin guano, Mackellar Islets. 19-12-13.
- VI. Ten penguin guano cultures.

All six specimens were secured within or close to Commonwealth Bay, in latitude 67° 0' south, longitude 142° 36' east, approximately.

#### *I.—Soil, near penguin rookery, containing guano, Cape Denison.*

19-12-13.

Gritty soil, containing small fragments of guano.

Plated on agar.

#### *Plate A—*

- (1) One small, round, creamy colony. Short, moderately thick, Gram-positive bacilli. Sporing forms, Gram-negative, stout and oval.  
Several minute golden colonies. Short, almost coccoid, Gram-positive bacilli.

## Plate B—

- (1) Two yellow colonies; a Gram-positive, almost coccoid bacillus.
- (2) Two creamy colonies; a Gram-positive, sporing bacillus showing degenerated forms.
- (3) Large numbers of minute brownish colonies. These were colonies of a Gram-positive, coccoid bacillus, and of a Gram-positive coccus. (B4.)

Subcultured on:—

	Agar.	Serum.	Gelatine.	Potato.	Broth.
A 1	Whitish growth...	Whitish, wrinkled pellicle.	Scanty down the track; a thin whitish pellicle on top surrounding puncture.	Fine honeycomb growth. Medium dirty grey.	.....
A 2	Fine growth ...	No growth visible	No change ...	No change. (Had probably died out.)	.....
B 1	Dense growth, with golden yellow tint.	Thick yellow growth, digesting the serum.	Scanty down the track; heaped up in centre of puncture, and spreading outwards. Yellow.	Yellow growth; medium has purplish tinge.	Faint turbidity.
B 2	White slimy growth.	Greyish white, slimy growth.	Scanty down the track; forming a whitish pellicle at the puncture with irregular edges and somewhat granular-looking.	Greyish raised growth.	Broth clear above.
B 3	Fine, white colonies.	Very fine growth..	Very scanty down the track; nil on top.	No change ...	Slight deposit. Broth clear above.
B 4	Creamy white growth.	Yellow growth, liquefying medium.	Scanty down the track, heaped up at puncture, creamy yellow.	Yellow growth ...	Turbid; no chain-formation.

## II.—Penguin Guano, Stillwell Island. 30-12-13.

Hard, dirty, pinkish-white masses of guano.

Plated.

A. Two creamy colonies. Gram-positive bacillus; sporing forms short, oval, and Gram-negative.

B. No growth. Plate later overgrown with mould from sides.

	Agar.	Serum.	Gelatine.	Potato.
A 1	Whitish, filmy growth.	White, wrinkled growth.	Scanty down the track: whitish, translucent, opalescent growth around puncture.	Grayish, raised growth, medium purplish grey.

III.—*Penguin Guano, Mackellar Islets.* 19-12-13.

Dirty-white, granular material; a few gull feathers mixed with it.

Plated.

A. One small, round, yellow colony; Gram-positive coccus.

B. Overgrown with mould from sides.

	Agar.	Serum.	Gelatine.	Potato.	Broth.
A	Yellow growth ...	Yellowish growth	Scanty down the track; yellow growth around puncture.	Yellow growth, medium grey.	Turbid; no chain-formation.

IV.—*Penguin Guano and Soil from a rookery, Cape Denison.*

19-12-13.

Grit with fragments of guano.

Plated.

A. Numerous creamy, round colonies.

Four colonies stained; all Gram-positive bacilli; subcultures, sporing bacilli; sporing forms shorter, oval and Gram-negative.

B. Overgrown with mould from sides.

All subcultures show the following cultural characteristics:—

*Agar.*—Abundant greyish-white growth spreading over the surface.

*Serum.*—Wrinkled, whitish growth, semi-translucent.

*Gelatine.*—Varying degree of liquefaction of the gelatine, forming a cup or funnel-shaped depression, the sides lined with a whitish growth.

*Potato.*—Greyish growth, medium dirty-grey colour.

V.—*Brine and a little Penguin Guano, Mackellar Islets.* 19-12-13.

Whitish, granular material adhering to the sides of the tube as if wet.

Plated.

A. One round yellowish colony; Gram-positive coccus.

B. One pinkish colony, and mould growing in from the sides. The colony is a Gram-positive "yeast."

	Agar.	Serum.	Gelatine.	Potato.	Broth.
A	Dense lemon-yellow growth.	Yellow growth ...	Scanty growth down the track; growth heaped up in centre of puncture, and spreading outwards, yellow.	Yellow growth, medium grey.	Turbid.
B	Pink growth ...	Bright pink growth.	Scanty down the track, heaped up at top, pink.	Pink, raised growth.	Turbid.

*VI.—Ten Penguin Guano Cultures.*

Ten cultures from guano were submitted, two of which, on agar, and labelled "Guano, Cape Hunter, Commonwealth Bay, 22-12-13 and 14-1-14," showed no growth, and were discarded. The remaining ones showed a growth of Gram-negative bacilli thicker than coliform bacilli, occasionally forming filaments, with central or subterminal elongated spores. The cultures were—one labelled "Penguin Guano," and the remaining, "Guano, Mackellar Islets." The culture from the first was on gelatine, and the remaining cultures were on agar slopes or stabs.

Subcultures were made on 20-8-14. One was overgrown with mould, one showed no growth, and the remaining six showed the presence of a Gram-negative sporing bacillus as indicated before. All these were subcultured on agar, serum, potato, and broth, and gave the same reactions on the different media :—

On agar, a whitish filmy growth.

On serum, a raised, spongy, greyish-white growth.

The growth on potato was not obvious, but produced a brownish discolouration of the medium.

On broth it formed a whitish, wrinkled film on the surface, with turbidity of the broth.

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## CHAPTER III.

## COMPARATIVE SUMMARY—BACTERIA OF MAMMALS, BIRDS, AND FISHES.

THE bacteriological observers of four previous Antarctic expeditions agree on the fundamental fact that the intestinal canal of an Antarctic vertebrate is relatively sparse in bacteria. It is sufficient to make smears from the contents in various situations roughly to note this phenomenon. To go further and make cultures demonstrates in many instances no sign of growth, where in more temperate regions growth would be invariable. Dr. Harvey Pirie adds, "that in a number of instances they (the intestinal tracts) appear to be altogether sterile, or, at all events, any bacteria they may contain fail to grow on the ordinary, commonly used nutrient media."

It is difficult to believe that the whole of the alimentary tract in any one animal should be free of bacteria, especially as the environment of ice, snow, soil and sea teem with micro-organisms, according to our limited observations. Certain it is that the bacterial flora varies in one respect; according to the kind of food which is digested. In the seals, which ingest variable quantities of fish, crustaceans, sea-water, surface plankton and debris from the sea-bottom, one would expect bacteria to take their usual part in the process of breaking down organic matter. Thus, in the stomach and intestine of the many seals, which were killed for food and for scientific purposes in Adelie Land, large masses of food, rich in protein, were found. In the lower intestine and rectum putrefaction seemed, with due account being taken of the climatic conditions, proportionately as active as one finds in the bowel of a warm-blooded vertebrate of a temperate country. Again, it is apposite to note that the stomach of seals is infested, almost without exception, with nematodes, and throughout the intestine cestodes abound in great numbers. External parasites, too, are exceedingly common. This generalisation may be made, too, of birds which feed on carrion, like the Southern skua gull and giant petrel. The skua gull is also a bird of prey. Many specimens of skua gull and giant petrel were examined, and internal and external parasites were recovered from them in almost every instance.

In the case of the penguins, sharing the characteristics of both fish and bird, one should make reservations. Gaseous putrefaction is present, but the protein food of the penguin does not need the same destructive metabolism. In the stomach of this bird one finds mostly crustaceans, with small fish, pebbles and organic debris. All the Antarctic penguins eat considerable quantities of snow during the summer breeding season. Occasionally in an Emperor penguin, fresh from the sea, the stomach is loaded with small whiting-like fish.

With regard to parasites, it was very common to examine two or three birds without finding a single specimen amongst the feathers. On the other hand, nematodes were always found in the stomach or intestine and, in the majority of cases, cestodes in the intestine. Occasionally in an odd example tape-worms would be found in hundreds right throughout the intestine.

Petrels frequenting the coast of Antarctica may be seen on the wing picking up their food on the blizzard-swept patches of open water, or amongst the pack-ice which encircles the whole continent. During the summer they come to land—a small outcrop of rocks on the icy coast, or an island—and in some crevice lay their eggs and rear their young. The birds to which particular reference is made are Antarctic, snow, silver-grey and Wilson petrels, though some species of prion and the Cape pigeon live practically the same life. One would expect these birds, living in such an aseptic environment, to be singularly free from bacteria. Actually their alimentary tract is very clean and free from any active putrefaction in comparison with the intestinal canal of a giant petrel or an Emperor penguin. One appreciates this fact after searching in many specimens for internal parasites. The food of the Antarctic and snow petrels is in the main crustaceans; reddish-brown masses of *Euphausia* which float with the streaming sludgy ice in leads of the pack-ice or in the open sea. Small fish have been occasionally found in the stomach, and doubtless these birds ingest diatomaceous matter and other surface plankton. Like penguins and seals, they have their external and internal parasites; but not in great profusion. It is easy to imagine that one may fail to inoculate a culture tube, when bacteria are scarce and the platinum needle touches only a few points in the relatively large area of intestine.

Reference has already been made to the fish inhabiting the coastal waters of Antarctica. According to Mr. E. R. Waite's\* description of the fishes of Antarctica, they appear to subsist on algæ (swallowed with the invertebrates), crustaceans and small fishes of the same species. Putrefaction is not apparent as a metabolic process, since it would have to occur at a temperature close to the freezing-point of sea-water (29° F. approximately). Internal parasites (nematodes in particular) live in myriads throughout the intestine, invading the muscles in some cases. Nematodes were often found in the liver of the fish in Adelie Land. At Macquarie Island the "cod" were not edible on account of their muscles being full of parasites. Of course bacteria are present; restricted in numbers. Mlle. Tsiklinsky, in the illuminating account of her investigations, has made findings of great interest. Two of our cultures from *Notothenia* gave positive results, and a living culture (a Gram-negative, sporing bacillus) survived from a fish taken in the deep-sea trawl at 1,700 fathoms. It was examined as a subculture in Australia.

To illustrate the disparity in the results obtained by bacteriologists in Antarctica, Dr. Harvey Pirie, of the Scottish National Expedition, sums up:—"Dr. Ekelöf obtained a bacillus twice from intestinal contents of skuas, but failed to get any growth from the same species on other occasions, and also could get no growth from Adelie penguins, gentoo penguins, terns (*Sterna hirundinacea*) or cormorants (*Phalacrocorax atriceps*). Dr. Gazert, from Weddell seals, crab-eating seals, and sea-leopards always obtained bacteria in the large intestine; more rarely in the small intestine and stomach. In the stomach and intestinal contents of the following birds, he found no bacteria, either by aerobic or

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\* Australasian Antarctic Expedition. E. R. Waite. Fishes. Series C., Vol. III, Part I, p. 22. Adelaide, 1916.

anaerobic cultivation:—King penguins, Adelie penguins, Antarctic petrels, snowy petrels, terns (*Sterna hirundinacea*), and a species of *Profinus*. Only from one tern and one Adelie penguin were growths obtained, and in neither case could fallacy from accidental contamination be excluded. Dr. Charcot reports that the examination of faecal matter from the intestines of various seals, birds and fishes showed the presence of numerous and various bacteria, in smaller numbers, however, than in temperate regions. He brought home a number of live cultures from seals, gulls, penguins, petrels, and fishes, from which Mlle. Tsiklinsky was able to isolate in pure culture twenty-four species of bacteria, of which fifteen could be identified with well-known forms, the others being apparently new species or varieties. Those from the fish, in particular, appeared to be very polymorphic and indefinite in their characteristics." As a preface to this highly interesting and important generalisation, made in 1912, Dr. Harvey Pirie makes a bare statement of his own findings:—"Growths of one or more species of bacteria were obtained from the alimentary tract of thirteen of the twenty species examined; from three of the four species of seals, and from ten of the fifteen species of birds."

We may add the following summary, from work on the limited field of animal life to which we had access:—

Cultures were successfully grown from the alimentary tract of nine out of thirteen vertebrates examined; from two species of seals, five species of birds, and from two species of fish. In addition one should add:—

- (a) Growths, yielding two organisms in pure culture, appeared from the nose and throat of both the Weddell and the crab-eater seal.
- (b) Many cultures were made from wounds of Weddell seals. Six original and two subcultures were carried back to Australia and examined there.
- (c) Examples of penguin guano, taken from six different situations in Adelie Land, yielded in Australia various bacteria; four organisms being isolated from one specimen.
- (d) Cultures made from a tern, a prion, a silver-grey petrel, a Ross seal, and from a holothurian, obtained in the deep-sea trawl, gave negative results.

To come down to details and to make comparisons, the findings in the case of seals have been positive and consistent. For example, Mlle. Tsiklinsky has tabulated a list of nine organisms recovered from the intestinal contents of Antarctic seals; five bacilli, three cocci and a sarcina. Their characters on various media have been minutely described and contrasted. This observer isolated three Gram-positive, sporing bacilli of various types, which, according to their biological and morphological characters, are to be placed in the group of *Bacillus subtilis mesentericus*. It may be stated that, as a result of our findings during 1912 in Adelie Land, bacilli of a type similar to *Bacillus subtilis mesentericus* were very frequently found; so frequently that the colonies were occasionally treated as real growths and not contaminations, since, in many instances, the culture tubes had been previously incubated for twenty-four hours without growth,

and rigid antiseptic precautions had been taken in making the inoculations. Sporing bacilli and moulds flourished in the Hut, even at a temperature below freezing-point. But so often, particularly in cultures from the rectum of the Weddell seal and skua gull and from frozen algæ, were fusing colonies (on agar) obtained of a dense, dry, adherent, wrinkled, white or brownish character, which was invariably a sporing bacillus, that we were inclined at length to regard it as a *bonâ fide* growth. Similar cultural appearances were noted by Mlle. Tsiklinsky. Dr. Harvey Pirie discovered very stout, round-ended, Gram-positive bacilli, arranged in pairs, end to end, in the intestine of a sea-leopard, but as they were in the smear from a growth of mixed bacteria the appearance of the colonies is not described.

With regard to the other two bacilli, found by Mlle. Tsiklinsky, one is a new species, and the other belongs to the *Bacillus coli communis* group. Dr. Harvey Pirie notes Gram-negative bacilli in the intestine of the Ross seal and the sea-leopard, and Dr. Gazert speaks of "slender bacilli" in the colon of the crab-eater seal and "bacteria" in the colon of the Weddell seal. As a result of an examination made in Sydney of coliform organisms, belonging to the Weddell seal, crab-eater seal and skua gull, and derived from cultures originally made in Antarctica, it was established, as a result of their reactions on five "sugars," that they fell into the Friedlander-Neapolitanus group. The lower intestine of the Wanderer albatross contained an organism showing the same reactions.

Among the cocci, Mlle. Tsiklinsky claims a new species in a large Gram-positive organism with fine, transparent colonies, and identifies, as well, *Staphylococcus albus cereus*. The third coccus is small and Gram-positive, secreting a lemon-yellow pigment. Dr. Harvey Pirie found Gram-positive cocci, both in the intestine of the Ross seal and in that of the sea-leopard, as well as Gram-negative bacilli showing bipolar staining. In the Bacteriological Log we have noted in mixed growths bacilli with bipolar staining, cocco-bacilli and cocci; and, since returning to civilisation, have grown an organism from granite sand following in almost every reaction Mlle. Tsiklinsky's pigment-secreting coccus. In Adelie Land, coarse granitic gravel, mixed with a little sand, together with slowly decomposing organic material, make up in certain localities on the shallow sea-bottom a mud which we often found mixed with pebbles in the stomach of Weddell seals. It is not surprising, therefore, that this coccus should have been present in seals' excrement gathered during Dr. Charcot's expedition. Again, a Gram-positive coccus, showing lemon-yellow growth, varying in slight particulars from the above, was present in frozen spray, incorporated with a little penguin guano, gathered on the Mackellar Islets, off the mainland of Cape Denison. The bacterium may have been derived from the intestine of an Adelie penguin or have been present in the sea-water. In case the latter were true, the organism may be easily accounted for in the intestine of a seal.

With regard to penguins, there is no lack of evidence to show that bacterial life is prolific in the alimentary tract. We were able only to make cultures from the Adelie

penguins inhabiting the rookeries within Commonwealth Bay, Adelie Land, but Dr. Harvey Pirie obtained results in the case of the Emperor penguin, the ringed penguin and the gentoo penguin. Mlle. Tsiklinsky does not specify the kind of penguin from which Dr. Charcot took his specimens.

Two Gram-negative bacilli, one of which is new to science, were isolated by the last-named observer. The known organism belongs to the *Bacillus pyocyaneus* group and was found to possess pathogenic properties; a fact of peculiar interest. From the Emperor penguin, Dr. Harvey Pirie obtained short and long bacilli, and in a mixed growth a Gram-negative coccus or cocco-bacillus and fine, motile bacilli emanating a strong faecal odour. The ringed penguin showed large, Gram-positive cocci and the gentoo penguin, Gram-negative cocco-bacilli and Gram-negative strepto-bacilli.

In Adelie Land, during a period when Gram's stain was not accessible, the following notes were made in the Bacteriological Log of bacteria grown in culture from the Adelie penguin:—(1) Rectum—short, stout bacilli with bipolar staining; (2) Rectum—long and short, stout and curved bacilli; (3) Rectum—long and short, stout bacilli; (4) Intestine—cocci and coliform bacilli; (5) Intestine—slender bacilli in short chains and coliform bacilli; (6) Intestine—long, stout bacilli; (7) Lower intestine—long and short, slender rods; (8) Frozen excreta—cocci and coliform bacilli; (9) Cocci and cocco-bacilli. In two instances, not noted in the Log, a heavy faecal odour was present in *glucose shake* cultures, which had become disrupted by the formation of gas-bubbles. From these rough observations, and the fact that putrefaction and gas-production are normal phenomena in the intestine of penguins, it seems highly probable that species of *Bacillus coli communis* inhabit the lower bowel, just as they do in the proven cases of the seals and skua gulls. Cocci may be ingested in the snow which the penguins eat during the Antarctic summer. Gram-positive cocci were frequently found in uncontaminated snow and ice.

Unfortunately the single tube showing growth, of four original cultures carried back to Australia, dried up before subcultures could be made. However, from six specimens of penguin guano gathered in various situations at Commonwealth Bay, an assortment of organisms was obtained at the Bureau of Microbiology, Sydney. On four occasions, Gram-positive, sporing bacilli (possibly of two species) were isolated; once a Gram-positive coccoid bacillus, three times, Gram-positive cocci (of two species at least) once, a Gram-negative, sporing bacillus, and once a Gram-positive "yeast." The Gram-positive, sporing bacilli, in three instances, were unique in having stout, short, oval, Gram-negative, sporing forms.

Actually these specimens of guano, although gathered in sterile test tubes, were not pure examples of dried penguin faeces. Gritty soil and small feathers were present in several of the tubes, as well as the dry, dirty pink or greyish flakes which are to be found caked together in old rookeries. Then, too, one should consider the contamination of bacteria from melting snow and from the organisms which stream down in the thaw-

water from higher levels of the rookery. So that, to make the most extreme supposition, bacteria from the scanty granite sand, lichen soil, moss soil, morainic mud, algæ, snow, ice and sea-water (frozen spray) may all have been added to those already existing in the guano.

Thus certain of the cocci may be accounted for, since they were often found in cultures made from ice, snow, algæ, granite sand and morainic mud, while "yeasts" were grown from ice, sea-water, lichen soil and moss soil. Finally, a Gram-positive cocco-bacillus, similar in cultural characters to the above, was present in morainic mud.

One fact to signalise is that *Bacilli coli communis*, thermophilic in habit, do not seem to have survived in a single specimen of guano, and, as one might have expected, sporing bacilli have resisted the extreme cold (probably never lower than  $-40^{\circ}$  F.).

There is no dearth of bacteria in the intestine of the Antarctic skua gull. Dr. Ekelöf obtained a bacillus twice from this bird, and Dr. Harvey Pirie encountered a large Gram-negative coccus or cocco-bacillus and a Gram-negative bacillus showing spindle-shaped involution forms. Mlle. Tsiklinsky, in an Antarctic "gull" (presumably *Megalestris antarctica*) found a short, thick, Gram-positive bacillus of a new species, and small, Gram-positive staphylococci. The notes from our Bacteriological Log distinguish in cultures:—(1) Bacilli of a coliform type both in the small intestine and rectum; (2) long and short, stout bacilli, some in short chains, some sporing; (3) long and short, stout, sporing bacilli; (4) small, slender, sporing bacilli; (5) cocco-bacilli. Later, in Australia, eight growing cultures were investigated, with the result that a coliform organism of the Friedlander-Neapolitanus group was recognised, and sub-cultures isolated a large Gram-positive bacillus.

Thus the bacteriological researches of four expeditions have demonstrated in the skua gull at least seven different organisms (including bacteria of a coliform type and sporing bacilli). Unfortunately in only three instances have the cultural characters of these bacteria been more or less fully worked out. Enough, however, has been discovered to leave an ample field of investigation for a future student and to give him more than a hint of his probable findings.

One will not be surprised that the petrels of Antarctica have yielded a scanty flora, and, in illustration, we merely quote some negative results.

Dr. Harvey Pirie failed to obtain growth in four instances. Dr. Gazert found nothing in aerobic and anaerobic cultures from the Antarctic and snow petrel: in cultures made in Adelie Land and carried back to Australia (two from the Antarctic petrel, three from the silver-grey petrel, and four from the snow petrel) there was a fine growth in one tube, but the organisms had died by the time they were examined in Sydney. In this connection, it may be mentioned that seven inoculations we made from a new species of prion produced no colonies, and of two from a tern, one gave a fine growth which did not survive. Dr. Harvey Pirie records a similar experience with Cape pigeons, Wilson petrels, terns, and sheath-bills; all birds which frequent the Antarctic zone.

In a mixed growth from a giant petrel, Dr. Harvey Pirie found Gram-positive cocci and Gram-positive and Gram-negative bacilli. In common with Mlle. Tsiklinsky, he mentions a large, Gram-negative cocco-bacillus, and the former adds a Gram-positive, sporing bacillus of the *Bacillus subtilis mesentericus* group.

We may summarise the notes of cultures from Antarctic and snow petrels in the Bacteriological Log:—*Lower Intestine*—(a) Cocci and cocco-bacilli; (b) long and short bacilli; cocco-bacilli; (c) cocci; (d) staphylococci (white in culture). *Rectum*—(a) Staphylococci (white in culture). *Small Intestine*—(a) Large cocci and cocco-bacilli; (b) cocci; (c) cocco-bacilli; (d) slender bacilli; (e) large cocci; (f) a “yeast”; (g) cocci; (h) a “yeast.” Thus the large cocco-bacilli re-appear once more, while Dr. Harvey Pirie has isolated from the intestine of a snow petrel Gram-positive staphylococci (white in culture), which are probably identical with those we found in the rectum and lower intestine of the Antarctic petrel.

No observer has yet claimed a species of *Bacillus coli communis* in the intestine of these petrels, though it is highly probable that they exist in the carrion-feeding giant petrel. It will be a point of no common interest definitely to establish whether these sea-birds of the southern high latitudes harbour bacteria of a coliform type.

From the excrementa of Antarctic fishes Mlle. Tsiklinsky has isolated a large number of bacteria with a marked tendency to polymorphism. Five bacilli, separate in cultural characters, exhibit involution and change of form in such an extraordinary degree that this observer thought at first that her cultures were impure:—“L’observation microscopique des préparations, faites avec des cultures préalablement isolées à plusieurs reprises, nous a donné d’abord l’impression que nous avions affaire à des cultures impures et que la nécessité de continuer l’épuration s’imposait. Après quelques expériences de ce genre, nous avons cependant conclu qu’il s’agissait des microbes tendant fortement au polymorphisme.” Again, experiments showed a number of common characters; they are readily stained by aniline dyes, they conserve their stability during many months and do not need to be subcultured, they do not form spores, they grow well in media containing as large a proportion as 3 to 4 per cent. of sea-water salt and sodium chloride, and at a temperature in the vicinity of 0° C.

It appears that Fischer in his researches on the bacteriology of sea-water and marine mud—prior to those of Mlle. Tsiklinsky—isolated bacilli with a strong resemblance to the above, and concluded in his generalisation that they had an extraordinary tendency to assume polymorphic forms; further, that they grew well on media containing 3 to 4 per cent. of the salt of sea-water and at a temperature close to 0° C.

Mlle. Tsiklinsky includes in her tabulated list of bacteria found in the excrement of fishes, small and large, Gram-positive staphylococci and two species of “yeasts” which were found to flourish in media containing 3 to 4 per cent of marine salt and at a temperature of 0° C.

Dr. Harvey Pirie records negative results in two cultures from the intestine of *Notothenia coriiceps*.

Dr. Gazert found bacteria (no nitrifying or denitrifying organisms) on the slime of the skin of a *Notothenia*, and on one occasion obtained growth from four samples of the contents of stomach and intestine. Again, from a species of *Lycodes*, bacteria were again cultivated from the slime of the skin and were present in the contents of the stomach. No nitrifying or denitrifying organisms were grown.

The Bacteriological Log notes a few slender bacilli, long and short in one culture from the intestine, and large cocci in staphylococcal masses and some cocco-bacilli in another culture from the rectum of *Notothenia coriiceps*. Later, eight cultures from fish were examined in Sydney; four of these showed growth. Slender, Gram-negative bacilli and larger, Gram-negative bacilli forming a (?) mycelium appeared in a culture from the rectum of *Notothenia coriiceps* and slender, Gram-negative, sporing bacilli from the rectum of *Chalinura ferrieri*, recovered by the deep-sea trawl at a depth of 1,700 fathoms. The growth on agar, in either case, was fine and did not thrive in sub-cultures.

Finally, reference should be made to the indirect evidence supplied by smears made from intestinal contents of mammals, birds and fish (Chapter I). It is apparent from this list that bacteria were fairly numerous in almost every smear examined; from the stomach, small intestine and rectum of various species.

Coliform bacilli and Gram-negative, sporing bacilli were seen in the intestine of the Weddell seal, sea-leopard, giant petrel and skua gull. The bacterial content in each instance was high.

Probably the Emperor penguin and the Adelie penguin harbour organisms similar in species to *Bacillus coli communis*.

As to the petrels—Antarctic, snow and Wilson petrels, and a species of prion—bacteria are certainly present, in reduced numbers. Gram-positive micrococci and Gram-negative cocco-bacilli were found in every case, while Gram-positive and Gram-negative bacilli were often to be seen, and in the rectum of an Antarctic petrel there were bacteria very similar to *Bacillus coli communis*.

In the codfish there was an interesting assortment of organisms; their number being fairly high in the intestine.

Almost without exception, Gram-positive cocci or micrococci occurred in smears from animals of ten species.



## CHAPTER IV.

## CULTURES FROM ICE, SNOW, SOILS AND MARINE MUD.

THE following observations are extracted, verbatim or in summary, from the records of the Bacteriological Log :—

## ICE (INCLUDING FROZEN ALGÆ AND FROZEN SEAWEED).

*Frozen Algæ.*

9-5-12. Frozen algæ from a thaw-pool on a rocky ridge 150 feet above the sea were secured. This particular situation was not frequented by penguins. The ice had a dirty greenish tinge.

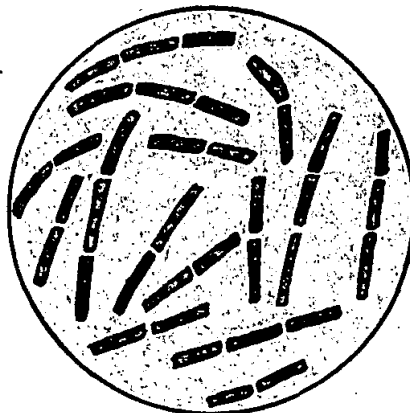
On thawing out a small fragment on a slide and putting on a coverglass, many filamentous, green algæ, diatoms of various forms, protozoa and bacteria (cocci and bacilli) were seen. A smear was stained by Leischmann's method.

A chained bacillus was observed, non-motile, with nodular, deeply stained areas, in the form of filaments of varying length consisting of rods, oblong and block-like in outline, approximated end to end. There were a few micrococci and diplococci; no rotifera.

11-5-12. A *serum agar slope*, after 60 hours at 37° C., shows a pale, glistening, somewhat waxy growth spreading slowly. Numerous bacilli, long, short and a few chained.

After 72 hours at 37° C., this culture became infected with mould and was discarded.

25-5-12. A cover-slip preparation was again examined. Besides algæ, diatoms, infusoria and rotifera, there were bacilli in large numbers, some single, short and rod-like, others longer and chained. The short, single bacilli were capable of more active movement than the long forms. Propulsion, flexion and straightening were executed; in the case of the first-named, by the aid of minute cilia or flagella observed with a  $\frac{1}{10}$  inch objective.



Methylene blue was allowed to run under the cover-glass and the bacteria and other micro-organisms immediately took up the stain. The chained bacilli were characteristic in appearance. They had very distinct, nodular dark markings, producing in some cases a spotted or barred appearance.

25-5-12. Cultures were made on various media. To prevent contamination, the following method was used:—Small lumps of ice, freshly procured in a sterilised canvas bag, and at an outside temperature of 10° F., were dipped with sterilised forceps into boiling water until they had almost thawed away, when the pieces were dropped into culture tubes of serum agar, nutrient agar, glucose agar and gelatine.

Two of these cultures became infected by a wrinkled growth which recalled *Bacillus subtilis* and were discarded\*. The *gelatine*, which was sloped, had a white growth; cocci in staphylococcal masses, and slender, bacillary rods ( $4\mu$  to  $7\mu$  in length) in short chains, with deeply stained areas.

The *serum agar* culture was examined after eight days. The lower part of the medium was commencing to liquefy, but on the upper part there were a few, minute, white, papilla-like colonies. Cocci, motile,  $1.8\mu$  in diameter, discrete and adhering in short chains. Bacilli, motile, in the form of slender rods varying from  $2.8\mu$  to  $9.7\mu$  in length; many curved or aggregated in short chains, apparently sporing. Coccoid or oval bacilli were also present,  $2.1\mu$  in length.

8-6-12. A cover-slip preparation of frozen algæ showed actively motile bacilli,  $2\mu$  to  $4\mu$  in length, chained bacilli, micrococci and diplococci.

A *serum agar* culture (after four days at 20° C.) had a whitish, wrinkled, somewhat adherent growth. Cocci, and diplococci with bacilli ( $3\mu$  in length on the average) showing short chains. Subcultures were made.

After three days at 20° C., a *serum agar slope* has become covered with a white wrinkled, adherent growth.† Bacilli in rods, about  $3\mu$  in length, and  $7\mu$  in width, chains or two or three.

15-6-12. A culture of frozen algæ on a *gelatine slope* (after three days at 20° C.) appears as a filmy, greyish growth with much liquefaction of the medium. Short, stout bacilli, slender, chained bacilli, cocci and diplococci.

19-6-12. A cover-slip preparation of frozen algæ (stained by Gram's method) shows Gram-positive cocci and diplococci, Gram-negative, chained bacilli and a few Gram-positive bacilli.

17-9-12. In a cover-slip preparation were observed diatoms, unicellular algæ and bacteria. The film was stained by Gram's method and counter-stained with carbol fuchsin. Gram-positive cocci and diplococci, associated with numerous Gram-negative bacilli.

\* In view of the results of Mlle Tsiklinsky's researches, these cultures may have been *bonâ fide* growths of a species of *Bacillus subtilis mesentericus*.

† Very probably the Gram-positive, sporing bacilli found in Australia to grow constantly from dried algæ.

*Frozen Seaweed.*

18-6-12. The specimens of frozen seaweed were obtained near the bottom of an ice-shaft, 12 feet deep, which was sunk in the glacier some 30 or 40 yards from the sea. In the cross-section of the ice, from above downwards, one could observe the transition from a structureless, bluish zone through a more stratified and crystalline, deep blue layer, to a yellowish and dark brown stratum near the bottom. Pieces of ice chipped from the last-named area were seen to contain pinkish and deep brown masses of weed; and there was a strong, saline odour like decaying seaweed, most noticeable when one stood in the bottom of the shaft.

A small fragment of the ice was thawed out, and under a cover-slip was seen to contain protozoa and numerous bacteria; motile bacilli (some chained), micrococci and diplococci. Stained by Gram's method and counter-stained with carbol fuchsin, the bacteria were seen as Gram-positive cocci and diplococci, short, stoutish Gram-negative bacilli and longer, Gram-negative bacilli.

19-6-12. Another smear from frozen seaweed contains numerous Gram-positive cocci and diplococci, long, stout, Gram-negative rods and more slender, Gram-positive bacilli.

After 48 hours at 18° to 20° C., there is a pale liquid growth, somewhat yellowish in tint and viscid in consistency, along the course of a stroke on *serum agar*. Gram-negative bacilli ( $3\mu$  to  $5\mu$  in length and  $7\mu$  in width); a few long and curved.

A *gelatine* culture appears as a pale, glistening growth with a fringed edge; no liquefaction observed. Gram-negative, slender bacilli of varying length; some long and curved; a few short chains.

A subculture on *glucose agar* (grown at 37° C.) has a greyish white, luxuriant growth of cheesy consistency; bacilli, short, long and curved.

An anærobic culture on *glucose agar* shows no perceptible growth, though gas bubbles have disrupted the medium.

*Morainic Ice.*

The above term is applied here to specimens of ice gathered in the vicinity of the terminal moraine. The glacier ice in this situation is guttered by channels in which runs thaw-water during the summer time. This water is discoloured by the mud which is borne by the glacier. Everywhere there are particles of grit and sand and accretions of mud and rock scattered throughout the blue ice.

6-7-12. In the smear from a cover-glass preparation were observed protozoa, Gram-positive cocci and diplococci, and a few Gram-negative bacilli (none longer than  $4\mu$ ).

Glacier ice, one quarter of a mile south of the Hut, in which there were embedded a few stones and pebbles, contained, besides protozoa, Gram-positive cocci and diplococci and a few Gram-negative bacilli.

After 24 hours at 18° to 20° C., on a *serum agar slope* there are discernible, growths of two kinds:—

- (a) Discrete, circular, white colonies.
- (b) A wrinkled, adherent, whitish growth spreading rapidly and drying in the centre; identical with a growth made from frozen algæ and described on 8-6-12 in a footnote as probably Gram-positive, sporing bacilli.
- (a) Cocci and diplococci in masses.
- (b) Stoutish bacilli of varying size, mostly about  $3\mu$  to  $7\mu$ , some slender, some in short chains.

A *glucose shake* culture is full of gas bubbles which invade and break up the medium; fine dot-like colonies. Bacilli of varying size similar to (b).

*Glacier Ice (Magnetic Shaft and Cave.)*

Specimens of glacier ice were procured from a shaft and cavern cut into the steep slope of the glacier, 1,105 yards south-south-east of the Hut, and at an altitude of 300 feet above the sea, in a place where the chance of contamination from guano or morainic mud was very remote (Plate 20). The prevalent wind, averaging 50 miles per hour throughout the year, blows almost constantly from the south, and the terminal moraine is at least 900 yards down the glacial slopes. In this situation the ice is sky-blue in colour and wind-swept. The fragments of ice were chipped out of the wall of the shaft, 6 feet below the surface—the ice here is of a deep azure colour—and carried to the Hut in sterilised canvas bags. The air temperature outside was well below zero Fahrenheit.

8-7-12. A cover-slip preparation shows small motile infusoria, some vermicular organisms and a few bacilli, one with bipolar markings (when the slide was stained).

Cultures were made on agar slopes, previously incubated, for 48 hours, as one batch of tubes had to be discarded owing to contamination by mould. From 8th July, 1912, onwards, culture tubes were incubated for 24 hours or longer, prior to making inoculations.

11-7-12. A *serum agar* culture, after 24 hours at 37° C., has a single, pale, circular colony. Cocci and diplococci in staphylococcal masses.

An *agar slope* shows six small, pale, circular colonies, after 24 hours at 37° C. Cocci and diplococci in staphylococcal bunches.

On another part of the slope is a greyish white, pitted growth with liquid drops on its surface. A stout, chained, sporing bacillus (length of separate bacilli,  $2.17\mu$  to  $4.34\mu$ ), associated with a slender chained bacillus, non-sporing.

On a *glucose agar slope* there is a rapidly growing, pitted, greyish growth with a tendency to form liquid drops on the surface. A chained, sporing bacillus similar to that found in the previous culture. Subcultures were made.

12-7-12. *Serum agar slope.* After 48 hours at 37° C., there is well-marked growth appearing along the course of two strokes, in the form of dull, opaque, light brown smears, corrugated and pitted. Chained, sporing bacilli.

*Glucose agar stab.* Numerous air-bubbles along the course of the stab with a somewhat flocculent growth; pellicle of dense pitted growth. Chained, sporing bacilli and yeast torulae.

An *agar stab* shows air bubbles along the stab and flocculent growth. Chained sporing bacilli.

An anaerobic subculture became contaminated by mould.

20-7-12. The *glucose agar stab* (11-7-12) has been covered by a viscid growth like blanc mange, in appearance, which caps the stab and upper part of the medium. A Gram-positive "yeast" and mycelium.

5-8-12. To preclude any chances of contamination from surrounding objects, an excursion was made to the cave for magnetic observations on the glacier, 300 feet above the sea, in order that culture tubes might be inoculated on the spot instead of of the ice being carried, for this purpose, to the Hut in sterilised canvas bags or glass tubes. Various precautions were taken.

The culture tubes had been previously incubated for 24 to 36 hours without any signs of growth appearing. These with a spirit lamp, platinum needle and a pair of forceps were taken up the glacier to the cavern. Outside the entrance a good deal of drift-snow had collected, and several culture tubes were inoculated with particles of snow. Inside the cave the spirit lamp was lighted, and the point of an ice-pick sterilised by heat. A deep cleft was then cut in the blue ice, which shelved and broke away in large masses. The ends of the forceps were sterilised by heat, and minute chips of ice were transferred to culture tubes of *serum agar* and *nutrient agar*, sloped.

The media had frozen, of course, as the air temperature stood at -15° F.; the velocity of the wind outside being 50 to 60 miles per hour. The media thawed out once more when they were replaced in the incubator, but, owing to the dryness of the air, there was very little water of condensation.

It may be of interest to note that amongst the ice and snow in the vicinity of the Hut and the rocky outcrop of Cape Denison small gritty particles of granite sand and smaller foreign bodies, just visible with a hand-lens of six diameters, are sparsely distributed. With the exception of penguin guano, generally caked or in small flake, when dried, and a small amount of morainic mud as dry dust *in situ*, there is nothing to contaminate the glacial slopes south of the Hut beyond the falling snow containing dust, and, possibly, free dust-motes which have travelled by lofty air-currents from a lower latitude. The wind of high velocity, comparable to a torrent of air, apparently rushes down from the continental plateau year after year. It is true that there are

short periods of calm when gusty winds and occasional whirlwinds come from the north. And in the summer, of ten to twelve weeks' duration, light north-westerlies may prevail for a day or more; but such occurrences are the exception.

So that beyond the infrequent whirlwinds, which track about irregularly, there is no aerial agent which could grossly contaminate the upper slopes of the glacier south of Cape Denison. And it may be noted in this connection, that after a methodical examination with a hand-lens of six diameters over the surface of the glacier south of the Hut, one could positively say that foreign bodies were distributed sparsely up to and a little beyond the terminal moraine, but that, on passing a level of approximately 150 feet and mounting the first steep rise, foreign bodies could not be seen. It should be remembered, of course, that the ice in the vicinity of the terminal moraine—about 100 feet in thickness—consists of basal strata which have passed over the underlying land, for, maybe, hundreds of miles, and are therefore dotted with specks of soil, particles and lumps of rock. The upper strata have travelled, possibly for great distances, always in a slow transition from compacted, fallen snow through the stage of *névé* to blue ice, and always moving with the slow northward surge of the continental glacier.

7-9-12. *Magnetic Cave*.—Ice cultures were examined, after 48 hours at 37° C.

*Agar slope*.—Doubtful minute colonies.

*Serum agar slope*.—No growth.

*Glucose agar slope*.—Several doubtful, elevated, pale colonies.

After 96 hours at 37° C.:—

*Agar slope*. Fine, pale colonies fusing. Slender bacilli up to  $4\mu$  in length; micrococci and diplococci.

*Serum agar slope*.—Greyish white colonies fusing in a fine growth. Slender bacilli up to  $4\mu$  in length.

*Glucose agar slope*.—Minute greyish colonies. Slender bacilli.

Examined a few days later, the *serum agar slope* was found to contain slender bacilli as well as a "yeast."

No further growth occurred and subcultures were not made.

15-7-12. *Glacier Ice* (200-300 yards south of the Hut).—Cover-slip preparations of surface ice show cocci and diplococci, motile bacilli and protozoan organisms.

*Glacier Ice* (500 yards south of the Hut).—A cover-slip preparation of surface ice shows motile bacilli of varying size, oval organisms like a "yeast," cocci and protozoa.

7-9-12. *Glacier Ice* (1,000 yards south of the Hut, altitude about 300 feet).—In a cover-slip preparation of surface ice there are yeast-like organisms, protozoa, numerous actively motile bacilli ( $2\mu$  to  $6\mu$  in length), slender and stout, straight and curved, and cocci and diplococci (encapsulated and motile).

7-9-12. *Glacier Ice* (1,100 yards south of the Hut, altitude about 300 feet).—Cultures, previously incubated, had been made on the previous day from surface ice.

*Glucose agar slope* (1).—A fine, somewhat reticulated, greyish white growth. Cocci and diplococci.

*Glucose agar slope* (2).—A pale, glistening smear. Cocci and diplococci in staphylococcal masses.

9-9-12.—The reticular, greyish white growth has spread. Cocci and diplococci, with short, stout bacilli.

No subcultures were made.

17-7-12. *Glacier Ice* (1 mile south of the Hut, altitude 600-700 feet).—A cover-glass preparation shows encapsulated micrococci and diplococci, protozoa, yeast-like bodies, but no bacilli. Culture tubes, previously incubated, were inoculated.

*Serum agar slope*.—After 24 hours at 37° C., minute, pale, circular growths were seen. After 48 hours appeared (a) pale, circular colonies growing slowly, as well as (b) a greyish white, fine, fused growth.

(a) Gram-positive cocci and diplococci in staphylococcal masses.

(b) Gram-negative, rather slender, chained bacilli.

17-7-12. *Glacier Ice* (2 miles south of the Hut, altitude 1,000 feet).—A cover-glass preparation of surface ice shows encapsulated cocci and diplococci and numerous bacilli, some slender and short, others stout and constricted so as to be like diplococci in appearance, while a few are longer and curved. The bacilli and diplococci are motile. The protozoa, stained with methylene blue, have a faint nuclear structure.

*Agar slope* (previously incubated) has (a) a few pale, circular colonies on the upper part of the slope, and (b) an adherent, pitted, whitish growth.

(a) Short, stout, Gram-negative (?) bacilli or cocco-bacilli; the constricted and dividing forms being very similar to diplococci.

(b) A Gram-negative stout bacillus showing short and long forms. *Note*.—Probably the Gram-positive sporing bacillus of frozen algæ which tends to retain Gram's stain only in early cultures.

18-7-12. *Serum agar slope*.—Glistening white, small, circular colonies. Gram-positive micrococci and diplococci, single and in staphylococcal bunches.

19-7-12. A subculture from the *agar slope* of (a) shows on *serum agar* a pale, fine smear growing slowly. A Gram-negative (?) short bacillus or cocco-bacillus; some forms being very similar to a diplococcus; maximum length, 2.17 $\mu$ . This culture extended slowly for a few days and then dried.

Subcultured on *serum agar* and *glucose agar*, this growth extended in much the same way. It was visible as a greyish white, fine smear, with a fringed periphery: Separate, circular growths were pale white in the centre, with a dull, fringed circumference.

Several later subcultures became infected with mould.

1-8-12. One *agar slope* subculture has a pale, fine growth similar to the preceding and several pale yellow drops on the surface of the medium. The latter stains as a Gram-positive "yeast."

16-9-12. *Glacier Ice* (5 miles south of the Hut, altitude 1,500 feet).—A sledging party returned with specimens of ice in sterilised canvas bags. These had been procured while digging a sub-glacial cavern, "Aladdin's Cave," 5 miles south of Winter Quarters, at a height of 1,500 feet above sea level. Among the specimens were some chipped from the surface and others from depths of 4 and 7 feet. Cover-slip preparations were made from them and culture tubes inoculated immediately after the party had arrived. It should be mentioned that "Aladdin's Cave" is at an altitude where the steeper glacial slopes give place to a more level expanse of blue ice, mounting in long waves for a distance of 3 miles, approximately, to the sastrugi fields which rise gradually towards the poles. The south magnetic pole, at an altitude of about 6,000 feet, lies some 350 miles to the south-south-east; the south geographical pole at an altitude of 10,000 feet, about 1,500 miles true south. As proved by later explorations of the Australasian Antarctic Expedition in the vicinity of the south magnetic pole, and in an eastward and westward direction, there are apparently no rocky exposures in the form of nunataks from which soil containing bacteria may be scattered by the wind over the surface of the plateau. The mean annual temperature at "Aladdin's Cave" is below zero Fahrenheit, and the annual wind velocity is more than 50 miles an hour. The thickness of the ice is probably not greater than 120 feet.

*Surface Ice*.—On thawing out a small fragment of ice, one could observe with a  $\frac{1}{8}$ -inch objective a great profusion of living organisms; protozoa, yeast-like bodies, globular, spindle-like or oval in shape, cocci and diplococci (encapsulated and motile), and actively motile bacilli, some long, slender and curved; others short and slender, and a few short, stout, and somewhat constricted.

16-9-12. *Ice* samples from a depth of 4 feet and from a depth of 7 feet contain protozoa showing nuclear structure, yeast-like bodies staining uniformly, Gram-positive micrococci and diplococci, and Gram-negative bacilli of various forms.

Bacteria, protozoa and "yeast" organisms are much more numerous in the surface ice.

17-9-12. *Surface Ice*.—

*Glucose agar slope*. After 24 hours at 37° C., a pale, fine growth is visible.

3 *A serum agar slope* (at 37° C.) has become contaminated by mould, and is discarded.

*Agar slope*. Doubtful growth after 24 hours at 15° to 18° C.



*Ice Four Feet below the Surface.*—No growths in two culture tubes; one inoculated at 37° C., the other at 15° to 18° C.

*Ice Seven Feet below the Surface.*—

*Glucose agar slope.* Doubtful fine colonies after 24 hours at 37° C.

*Agar slope.* A pale, fine growth.

*Glucose agar slope* (at 15°–18° C.). Discrete, circular, greyish white colonies.

19-9-12. *Surface Ice.*—

*Agar slope* (at 15°–18° C.). Fine, greyish white growths. Gram-positive cocci and diplococci.

*Glucose agar slope* (at 37° C.). A pale white smear spreading quickly. Gram-positive cocci in staphylococcal masses.

*Ice Four Feet below the Surface.*—

*Serum agar slope* (at 15°–18° C.). No growth.

*Serum agar slope* (at 37° C.). No growth.

*Ice Seven Feet below the Surface.*

*Glucose agar slope* (at 15°–18° C.). A large number of minute, pale, circular colonies. Gram-positive diplococci.

*Glucose agar slope* (at 37° C.). Very fine, greyish white, circular (?) colonies. Gram-positive cocci, large and small (micrococci).

*Agar slope* (at 37° C.). Fine, greyish white, fringed growth. Gram-negative bacilli, some short, stout and coccoid, others longer, swollen and involuting, interspersed with a few gram-positive cocci.

This culture, three days later, showed marked involution forms, long and curved, of a Gram-negative bacillus.

There was no opportunity to make subcultures from the above growths, which were watched until 22-9-12, when they had made very slow progress. All the culture tubes, before inoculation on 16-9-12, had been incubated for 24 hours at 37° C. without growth.

*Glacier Ice* (8 and 11 miles south of the Hut).—During a nine days' sledging trip in the month of September, 1912, I gathered specimens of ice at distances of 8 and 11 miles in a magnetic-south direction from the Hut. These were obtained from the surface, from a glacial cavern 6 feet below the surface, from compressed sastrugi snow and from ice-crystals taken from the wall of a crevasse. Small, sterilised, canvas bags, packed inside a larger sterilised bag, were used to collect the specimens, and sterile test tubes with cotton wool plugs were also used as receptacles. The mean temperature for the period of nine days was approximately 20° F. and the wind velocity from 55 to 60 miles per hour. The cavern—Cathedral Grotto—11 miles, 500 yards south of the

Hut, was about 1,800 feet above the sea on the gently rising plateau. The surface in the vicinity of the cave consisted of *névé*, localised patches of blue ice, and compressed sastrugi snow. Viewed with a hand lens of 6 diameters, the surface, whether of snow or of ice, showed the well-marked cleavage planes of crystals and doubtful fibrillary, and speck-like foreign bodies. I did not observe any, macroscopic in size.

Cover-slip preparations were made from each of the specimens, while, until cultures were inoculated, the bags were kept in the outer verandah, at a temperature always below zero Fahrenheit. Gram-positive micrococci and diplococci and small short and slender, Gram-negative bacilli were seen. The bacilli and diplococci were motile. No record is made of the presence of protozoa or yeast-like bodies.

Culture media, which had been previously incubated at 37° C. for 36 hours without growth, were inoculated.

21-9-12. *Glacier Ice (8 miles south of the Hut).*—After 84 hours an *agar slope* is covered with (a) fine, greyish white colonies, and (b) pale white, fine, circular growths.

(a) Gram-positive cocci.

(b) Gram-negative cocci-bacilli, associated with longer and more definite bacillary forms.

21-9-12. *Glacier Ice (11 miles south of the Hut).*—The following cultures were observed after 84 hours; some growing at 37° C., others at 18° to 20° C.:—

Two *serum agar slopes*. (a) Fine, discrete, greyish white colonies.

Two *agar slopes*. (a) Fine, discrete, greyish white colonies, and (b) pale white, circular growths.

*Glucose agar slope*. (a) Greyish colonies fusing in a continuous, fine smear.

*Gelatine slope*. (a) A few, fine, greyish colonies; no liquefaction noted.

In all four sets of cultures were found:—

(a) Gram-positive cocci.

(b) Gram-negative bacilli, short and coccoid, with longer, bacillary forms.

23-9-12. *Gelatine stab* subcultures of (a) and (b) appear as fine growths similar in appearance to the original cultures, with no liquefaction of the medium.

Several *gelatine shake* cultures have small, fine, flocculent growths in the medium, after 48 (?) hours at 18° to 20° C.; no liquefaction.

The original *agar slope* cultures of (b) show short, stout, Gram-negative bacilli and swollen involution forms.

25-9-12. A *serum agar slope* (after 8 days at 18°-20° C.) has a few (a) pale white, circular colonies and (b) a fine, greyish, fused growth, somewhat bluish in tinge.

(a) Gram-positive cocci.

(b) Short (in the main) Gram-negative bacilli.

*Névé* (50 miles west of the Hut).—A sledging party, under the leadership of Mr. C. T. Madigan, who returned to the Hut on September 26th, 1912, after a journey of two weeks to the west, secured specimens for bacteriological examination. Dr. L. A. Whetter took charge of several sterilised canvas bags and gathered pieces of surface *névé* (a transition stage between snow and ice) in a situation 25 miles from the sea, at a distance of 50 miles west of the Hut, and at an altitude of nearly 4,000 feet. During the latter half (25 miles) of the westward journey, the party's track lay over billowy sastrugi and fields of *névé*, rising gradually in long waves towards the south. The mean temperature, during the 14 days this sledging party was away from the Hut, was approximately  $-25^{\circ}$  F., and the average wind velocity registered at the Hut (at sea-level) was 58 miles per hour. On the plateau it must have been more than 60 miles per hour. The specimens were carried in canvas bags, placed in the instrument box on the sledge, and small fragments were examined immediately after the arrival of the party. The actual specimens were kept in a perfectly dry state in the outer verandah at a temperature below zero Fahrenheit.

7-10-12. Several grains of *névé* were thawed out on clean slides and the drops examined under cover-slips. There was the usual variety of micro-organisms previously seen in specimens of ice and snow from the plateau, except for the absence of protozoa and yeast-like bodies.

(a) Micrococci and diplococci, the latter apparently motile and some encapsulated.

(b) Bacilli; some rod-like (not more than  $3\ \mu$  in length), short, stouter forms and some curved; all actively motile. There were many bacilli immobile and clumped in zoogloea masses.

11-10-12. Three *agar slopes*, after four days at  $18^{\circ}$  to  $20^{\circ}$  C., show well-marked growth. (a) In two tubes there is a greyish, fine smear and a few white, circular colonies. (b) In the other tube there is a somewhat yellowish, central growth, and an outlying, greyish smear.

The greyish smear shows short, Gram-negative bacilli, many coccoid in appearance. The white, circular colonies are Gram-positive cocci.

*Glucose agar slope.* (a) A greyish smear, (b) discrete, circular, white colonies and a yellowish, circular colony.

(a) Gram-negative, short bacilli, many oval and coccoid in appearance;  $2.5\ \mu$  in maximum length.

(b) Gram-positive cocci.

14-10-12. The original cultures and subcultures of *névé* are progressing slowly at  $18^{\circ}$  to  $20^{\circ}$  C.

19-10-12. One *agar slope* subculture shows a greyish, fine growth with a fringed border. Coccoid bacilli showing involution forms.

*Lake Ice.*

In the deeper depressions among the rocky outcrops of Cape Denison are glacial lakes, the water of which is partly replenished in summer from the thawing ice and snow surrounding them. For about six weeks, or less, in the summer, the ice, which freezes to a depth of about six feet, thaws out. The ice itself is very clear and free from air bubbles; in fact, it is like solid glass with a pale-blue tint. Lichens, mosses, and algae flourish for a few months around the lakes and penguins occasionally visit them. All gross material in suspension sinks, and the upper layers of ice do not contain so many bacteria as are seen in the surface layers of glacier ice.

25-9-12. A cover-slip preparation shows relatively few micro-organisms; motile bacilli, short, long and curved, occasionally in zoogloea masses; micrococci, diplococci and zoogloea masses of cocci.

Several cultures were made from lake ice.

27-9-12. After 48 hours at 18° to 25° C., there is slight evidence of growth in two tubes.

30-9-12. *Agar slope.* (a) Small, white colonies set in a (b) greyish, fine growth.

*Serum agar slope.* (b) Greyish smear covering the lower part of the slope.

*Gelatine slope.* (a) A few white, circular colonies; no liquefaction of the medium noted.

*Glucose agar slope.* (a) Discrete, white, circular colonies and (b) a fine, greyish growth.

In the above cases appeared :—

(a) Gram-positive cocci in masses.

(b) Short, stoutish, Gram-negative bacilli, the shorter forms coccoid in appearance.

2-10-12. Growth has proceeded slowly in all the tubes. Smears give the same results as on 30-9-12.

*Sea Ice.*

Specimens were procured in a sterile test tube close to the shore, on August 30th, 1912. The meteorological conditions were :—Wind, 50 miles per hour, temperature, -26.5° F., with light drift.

In a cover-slip preparation there are numerous bacteria; motile (?) cocci (encapsulated) and motile, encapsulated diplococci, and short and long, motile bacilli.

A *serum agar slope* (1), after three days at 15° to 18° C., has a pale, viscid growth with a few outlying, circular colonies. Short, nodular bacilli.

*Serum agar slope* (2). Greyish, circular colonies. Short coccoid bacilli.

5-9-12. (a) *Glucose agar slope* and (b) an *agar slope* have each pale, fused growths spreading slowly. After (?) days at 18° to 20° C., these cultures were examined and there appeared, (a) bacilli, short and stout with a few longer forms, and (b) yeast torulae.

*Serum agar slope* (neglected), after 18 days at 12° to 15° C., is covered with a pitted, yellowish growth. No smears were made.

*Note on Glacier Ice.*

During the month of September, 1913, sterile specimens of ice were gathered from a shaft which was sunk into the blue ice of the glacier to a depth of 24 feet. The shaft was situated about 20 yards above the discoloured area where morainic material is embedded. Looking at cover-glass preparations of six specimens obtained from the surface, down to a depth of seven feet, one could roughly note that the micro-organisms present became fewer as one passed from the surface. Single free-swimming forms were numerous down to a depth of approximately three feet, and after that the bacteria were represented by clumps and zooglœa masses. Protozoa and yeast-like bodies were confined to the surface layers.

On October 9th, when the shaft had attained its greatest depth—24 feet—serial specimens were again examined.

*24 feet.* At this depth small pieces of soil and grit were visible in the clear blue ice and bacteria were numerous; free-swimming forms and zooglœa masses. A count of the bacteria was made on a hæmocytometer slide, and the approximate result was 118,720 micro-organisms per cubic millimetre.

*20 feet.* Ice blue and no foreign bodies visible. Hæmocytometer count, 19,040 micro-organisms per cubic millimetre. Free-swimming forms and zooglœa masses.

*12 feet.* Zooglœa masses very numerous, unduly increasing the hæmocytometer count, which gave roughly 1,703 micro-organisms per cubic millimetre.

*6 feet.* Zooglœa masses still numerous; 1,650 micro-organisms per cubic millimetre.

*4 feet.* Zooglœa masses still present; 1,093 micro-organisms per cubic millimetre.

*Surface Ice* (a few inches below the surface) containing protozoa and yeast-like bodies; 1,250 micro-organisms per cubic millimetre.

These observations are necessarily very approximate, and with partial contamination of the ice by morainic material no generalisations can be made. Still, there is the positive evidence of numerous bacteria in the ice, many of which in the thawed-out state regain their motility in a similar fashion to the rotifera.

*Snow.*

*Sastrugi Snow.* Freshly-fallen snow soon becomes compacted by its own weight, crusted over by the wind blowing at low temperatures, and then furrowed into wind-waves or sastrugi. The snow falls from the low altitude of a rain-cloud and may travel some distance, driven by a blizzard, before it settles on the ground. One would expect this snow to be free from organisms, but at the Hut in Adelie Land specimens were gathered from time to time, and in almost every instance were found to contain micro-organisms.

25-7-12. *Sastrugi snow* ( $\frac{1}{3}$  mile south-east of the Hut).

Cover-slip preparations of specimens gathered in a sterile Petri-dish show many motile bacilli, some slender and short, others longer and curved like a vibrio, while some are short and nodular. They are mostly in zoogloea masses, and even a single bacterium has a pale capsule. Micrococci and a lanceolate organism like a flagellated infusorian were also seen.

25-7-12. A basin (jelly-mould with a tightly-fitting lid) was sterilised in boiling water and dried with its lid on for more than half an hour in a very hot oven. It was then carried outside on to a high, rocky ridge. The air was charged with falling snow, which drove along in a wind of 50 miles an hour. The temperature was below zero, Fahrenheit. The lid of the basin was removed and some snow allowed to settle in the bottom, after which the lid was put on and the basin carried back to the Hut. Here small pieces of the sample were transferred on a platinum needle to a slide which had been previously heated in the flame of a spirit lamp. A sterilised cover-slip was put on the thawed snow, and the preparation viewed with a  $\frac{1}{6}$ -in. objective. Motile bacilli, short and slender, longer and curved, mostly surrounded by a pale capsule; zoogloea masses of straight and curved bacilli; no micrococci seen. The preparation was stained and a few zoogloea masses of cocci were observed.

27-7-12. *Sastrugi snow* ( $\frac{1}{3}$  mile south-east of the Hut).

*Agar slope.* After forty-eight hours at 37° C. are seen (a) a pale, fused growth at the lower part of the medium, and (b) a ridged and pitted mass above this.

(a) Gram-positive cocci.

(b) The bacteria were later found to be Gram-negative, sporing bacilli.

31-7-12. *Agar slope.* The pale growth has become lemon-yellow in tint like *Staphylococcus pyogenes citreus*. Gram-positive coccus.

3-8-12. *Sastrugi Snow* (obtained in a sterile Petri-dish 1,000 yards back on the slope of the glacier.—In a cover-glass preparation there are bacilli, micrococci, diplococci and protozoa (?).

*Falling Snow.* A cover-slip preparation shows encapsulated diplococci, short strings of cocci, single micrococci, a few segmented, motile bacilli and some zoogloea masses of bacteria.

7-8-12. *Drift Snow* (gathered 1,100 yards south of the Hut).

*Glucose agar slope* (previously incubated) shows several small greyish colonies, after twenty-four hours at 37° C.

*Serum agar slope* (1). No growth.

*Serum agar slope* (2). No growth.

These cultures became neglected owing to other work, and no smears were made from the first.

20-8-12. For the past few days there have been heavy falls of snow, with thick drift and blizzard wind. Fresh snow was gathered in a sterile vessel. Cover-slips preparations show micrococci, diplococci and bacilli, with zoogloea masses of bacteria; doubtful organic matter in the form of short cylinders, very like vegetable cells.

#### SOILS.

##### *Morainic Mud.*

Morainic mud at Cape Denison, Adelie Land, is the soil of the continent which has been carried a variable distance by the moving glacier, and deposited somewhere near the junction of morainic boulders and glacier ice. A number of samples of this deposit may represent soil from a dozen localities hundreds of miles from one another; just as the stony rubble of the moraine represents specimens which may typify half the continent of Antarctica. So the samples of morainic mud were precious as an epitome of the bacterial flora of a wide area. It is rational therefore that one may generalise from the results obtained.

That this soil will support vegetable life is evident from the following experiment:—During the spring of 1912 a small box of morainic mud, occurring as dry powder, was collected at the terminal moraine. Seeds of Federation wheat and English barley were sown in it, the soil being watered and the box kept inside the Hut close to where a maximum amount of light came through one of the roof windows. The seeds germinated and sprouted profusely to a height of 8 or 10 inches. The sprouts were yellowish-green in colour.

The falling slopes of ice behind the Hut in Adelie Land are furrowed-out by channels, down which thaw-water streams for the few weeks of the Antarctic summer. The ice itself is very discoloured, and wherever there are foci of rock, pebbles, grit or soil, thawing is most active, because of the absorption of heat by these foreign bodies exposed to the sun's rays. The soil, therefore, which has arrived full of its indigenous life, is contaminated—if such an admixture has not already occurred—by these rivulets of water containing the bacteria and other micro-organisms peculiar to the ice and snow. The soil when wet is dark or greyish brown in tint, but when winter comes and all moisture freezes, the mud is compacted into clods, which may dry in an atmosphere of low humidity into a bluish, friable powder. It was from this bluish powder that most of the specimens were taken.

22-8-12. A cover-slip preparation of a small particle of the dust in solution swarms with bacteria; Gram-positive micrococci and diplococci and Gram-negative, short, stout bacilli and longer rods. The bacilli and diplococci are actively motile; the micrococci seem motile to a lesser degree.

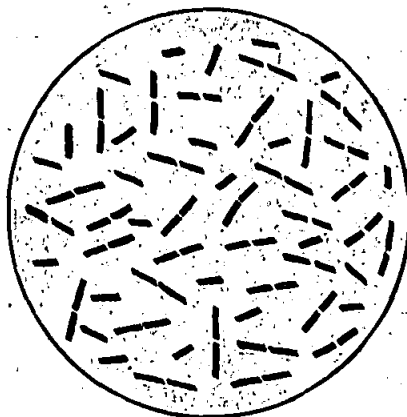
Cultures were made and grown at 18° to 20° C.

In three cultures of *gelatine* nothing is visible after 24 hours.

*Agar slope.* Very fine, greyish colonies growing slowly and commencing to fuse. Gram-positive cocci in masses, mixed with Gram-negative, short bacilli, some rather nodular in appearance.

26-8-12. In a *glucose shake* culture, after 72 hours at 18° to 20° C., there are, adherent to several minute particles of mud and scattered throughout the medium, many minute, pale colonies.

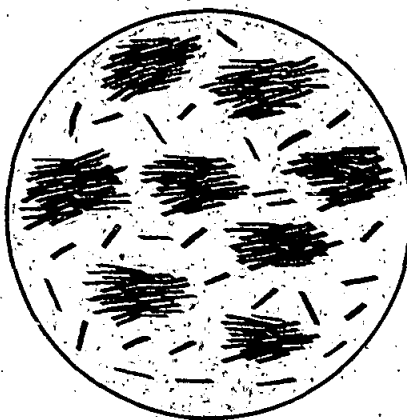
Three *gelatine* cultures. Spherical white growths in the medium and on the surface in one instance. No liquefaction of the gelatine. Small, short, blunt Gram-positive (?) bacilli.



*Serum agar slope* (?) (a) Several pale smears contiguous with minute particles of dust, and (?) (b) discrete, circular, white colonies.

(?) (a) Gram-positive cocci.

(?) (b) Gram-negative bacilli, as slender, straight rods:—



*Glucose agar slope.* Circular, white colonies growing slowly. Gram-positive cocci in staphylococcal masses.

*Agar slope.* Growing out from several minute particles of grit there are white, glistening smears. A fairly large, Gram-positive coccus.

29-8-12. Two of the *gelatine* cultures have become infected with the green mould—*Penicillium*.

From fine, white colonies on three other tubes subcultures were made and grown at 37° C.; no growth appearing after three days on a *serum agar slope*.



30-8-12. A subculture in *gelatine* of the Gram-positive coccus produced a white, glistening growth similar to the one described on the agar slope (29-8-12). No liquefaction of the medium.

5-9-12. Several culture tubes have become infected by *Penicillium*.

A *serum agar* subculture appears (a) as a white growth spreading quickly; and (b) an isolated brick-red colony.

(a) A coccoid bacillus showing involution forms.

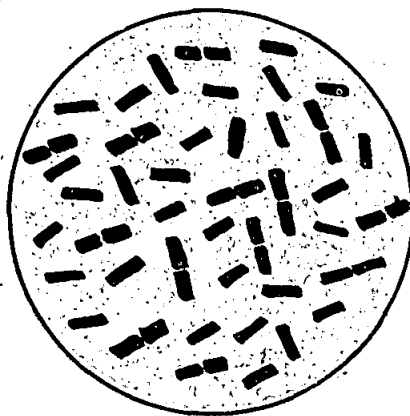
(b) Slender bacillary rods.

A *glucose agar* subculture of (a) shows a very fine, greyish smear with a fringed border (viewed with a lens). A coccoid bacillus showing involution forms.

#### *Macquarie Island Soil.*

A specimen was collected in a clean bottle and corked in December, 1911, during the stay of the "Aurora" at the Island on her first voyage to Antarctica. At the time there were no sterile tubes accessible. On Wireless Hill, approximately 300 feet above the sea, where the specimen was taken, the soil is dark and peaty. Skua gulls abound, but no penguins or sea-elephants are seen at this altitude. Macquarie Island is a lofty mountain peak pushing up from the bed of the ocean out of a depth of about 2,500 fathoms, in latitude 55° South, longitude 160° East, approximately. It lies almost mid-way between Antarctica and Australia—an isolated link of the volcanic chain connecting New Zealand and South Victoria Land—and its bacterial flora should form at some future time an interesting subject of research. The meagre results obtained from this specimen, roughly gathered, are recorded in the Bacteriological Log:—

5-4-12. A *gelatine* culture (after 48 hours at 15°-18° C.). Minute, greyish white colonies on the surface of the gelatine; slight liquefaction of the medium. Rod-like bacilli up to 5 $\mu$  in length.



*Glucose agar shake* culture (after 48 hours at 37° C.) shows numerous gas bubbles, which have disrupted the medium, and greyish growths. Bacilli, somewhat coliform in character; many with bipolar markings.

The gelatine culture afterwards became infected by an organism similar to *Bacillus subtilis mycoides*, and no subcultures were made from the colonies in glucose agar.

*Marine Mud.*

There was little opportunity to make bacteriological examinations of marine mud. In the boat harbour at Cape Denison dredgings were done during the winters of 1912 and 1913. Dark, rather strong-smelling mud came up with the dredge out of four fathoms of water. Later, when the "Aurora" sailed south on her final cruise, systematic trawling was done in depths up to 1,700 fathoms, in the vicinity of the Antarctic Circle. Specimens were collected and cultures made at various stations. These were examined at the Bureau of Microbiology, Sydney, and the results appear in Chapter V. A single record appears in the Bacteriological Log:—

October 3, 1913. A cover-glass preparation was made from mud of the boat harbour at Cape Denison; depth, four fathoms. Many bacteria present; cocci and motile bacilli of various types.

Several slides, which were stained by carbol fuchsin in Adelie Land, were, in Australia, decolourised by acid alcohol and re-stained by Gram's method. They show the following, in the specimen of marine sediment taken from the boat harbour at Cape Denison:—

Bacteria are in moderate numbers.

(a) Gram-negative bacilli ( $2.5\mu$  in length,  $.8\mu$  in width) with longer, curved forms; some are coliform in appearance.

(b) Gram-positive micrococci ( $.3\mu$  in diameter) and diplococci, in clusters.

(c) Slender, Gram-negative bacilli ( $2\mu$  in length,  $.2\mu$  in width).

## CHAPTER V.

## ANTARCTIC SPECIMENS.\*

## CULTURES SUBMITTED FROM SOILS, &amp;c.

*Ice.*

NINE cultures from ice were submitted. Three of these, labelled "17-1-14," "29th" and "21-12," showed no colonies and were discarded; the remaining six were on agar slopes and showed growth.

No. 1. 17-1-14 (?)—Whitish growth; Gram-positive "yeast."

No. 2. 5-1-14—A fine growth on agar; roundish oval, medium-sized coccus, Gram-positive, showing a tendency to lie in chains.

No. 3. 17-2 (?)—14—Pink colonies with a whitish growth around; Gram-positive "yeast," with cocci similar to No. 2.

No. 4. 17-2 (?)—14—Showed a fine growth of oval, Gram-positive cocci.

No. 5. 29th—Showed pink colonies of Gram-positive "yeasts" mixed with cocci as in No. 3.

No. 6. Fine growth of small cocci as in No. 2.

Subcultures of all the above were made on 9-3-14, as follows:—

No. 1. Showed pure cultures with yellow, Gram-positive "yeasts" forming around the colonies.

No. 2. Fine growth of Gram-positive cocci.

No. 3. Pink, Gram-positive "yeasts."

No. 4. Was overgrown with mould.

No. 5. Pink colonies of Gram-positive "yeasts."

No. 6. Fine growth of Gram-positive cocci.

Subcultures were also made on 5-6-14:—

No. 1. Gram-positive "yeasts" forming cream around the colonies.

No. 2. Very fine growth of scattered, Gram-positive cocci.

Nos. 3 and 5. Showed pink growth of Gram-positive "yeasts."

No. 6. Showed a scanty growth of a Gram-positive coccus.

*Summary.*—Three of the cultures showed the presence of "yeasts," two of them giving a pink growth on agar, one, a creamy yellow growth. Two cultures showed the presence of a Gram-positive coccus producing a fine growth. These two cultures of cocci died out in subsequent subcultures.

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\*The following report, compiled by Drs. J. B. Cleland, E. W. Ferguson, and K. Smith, is a summary of part of the work done on various specimens submitted to the Bureau of Microbiology, Sydney.

The "yeasts" from the ice were tested further on agar, serum, potato, and broth, with the following results:—

No. 1. Agar—A creamy, translucent growth with a pink tinge.

Serum—A creamy, whitish growth.

Potato—A yellowish growth.

Broth—A slight turbidity.

No. 2. Agar—Pinkish growth.

Serum—Pinkish growth.

Potato—Pinkish stain.

Broth—No turbidity.

No. 5. Agar—Scanty whitish growth, growing slowly.

Serum—White colonies growing rapidly.

Potato—No change visible.

Broth—No turbidity.

As the original of No. 5 showed pinkish "yeasts," these subcultures had probably become contaminated with some other organism.

#### *Moss Soil.*

Four cultures of moss soil were submitted.

No. 1, dated "17-12," showed a Gram-positive "yeast."

No. 2, dated "26-1," showed a Gram-positive (but poor), moderately elongated, moderately thick, sporing bacillus; spores elongated, and subterminal. The growth was heaped up and thickly wrinkled. Subcultures showed an even growth of fawnish-white colour and were dry and granular.

This culture was further tested, and on agar showed a whitish, honey-coloured looking growth; on serum, a whitish honey-coloured looking growth; on broth, turbidity with a white scum on the surface.

Nos. 3 and 4 showed the presence of a "yeast."

Nos. 1, 3, and 4 in the original showed a heaped-up, dull yellow growth. The subcultures showed fine, whitish colonies.

Re-subcultured on 20-8-14, Nos. 1, 3, and 4 showed the presence of a "yeast," and No. 2. showed the presence of a Gram-positive, sporing bacillus.

The "yeasts" were separately cultured on agar, serum, potato, and broth.

On agar they showed a scanty, muddy-coloured growth, spreading slowly; on serum, a fine growth; on potato, one produced a brownish-yellow, raised growth, the other two showed no visible change; there was no turbidity in broth.

Anaerobic cultures were made, with negative results.

#### *Morainic Mud.*

Six cultures, five on agar slopes and one on gelatine, made on 13th December and 16th December, 1913, and 14th January, 1914, all showed no growth and were discarded.

*Snow.*

No. 1, on gelatine, date undecipherable. No growth.

No. 2, 17-12, on agar slope. No growth.

No. 3. Discarded.

*Lichen Soil.*

Of eight cultures, four showed no growth and were discarded.

No. 1, dated 4-1-14, on agar slope, showed a pink growth.

No. 2. No date. Pink growth.

No. 3. 14-1. Agar slope. Pink growth.

No. 4. 17-2. Serum. Pink growth.

*Subcultures.*—Nos. 1, 3 and 4 showed no growth and were discarded. In No. 2 there were Gram-positive "yeasts." On agar, the subculture showed a fine, whitish growth; on serum, a whitish stain. No growth was produced on potato or broth.

Anaerobic cultures were made, with negative results.

*Mud from Sea Bottom.*

No. 1, 1,700 fathoms. Latitude 64° 34' South, longitude 127° 17' East. Temperature 0-3° C.; thick ooze and rocks. On gelatine. No growth.

No. 2, 17-2-14. 1,520 fathoms. Latitude 64° 37' South, longitude 108° 50' East. Agar slopes. No growth.

No. 3, 26-1-14. 300 fathoms. Latitude 65° 47' South, longitude 90° 39' East. Agar slopes. White growth.

No. 4, 12-1-14. 300 fathoms. Off Queen Mary Land. Agar slopes. Creamy growth.

Nos. 3 and 4.—Stained, these two cultures showed short, thickish, Gram-negative bacilli, often in pairs, showing a tendency to retain Gram's stain. In subcultures the growth was almost coliform.

These subcultures were not tested again until 10th August, 1914, and then showed the presence of a Gram-poor bacillus.

## MATERIALS FOR BACTERIOLOGICAL EXAMINATION.

In addition to the cultures submitted, a number of sealed test tubes were forwarded containing marine mud, for the purpose of bacteriological investigation.

The contents of these tubes were all treated in the same fashion, which may here be briefly described.

The tube was opened and a small portion of the contents emulsified in sterile tap water. From this emulsion, in each case, two agar plates were inoculated, the emulsion being thickly smeared over the surface. One plate was labelled "A" and the other "B."

From the same emulsion glucose broth tubes, covered with oil, were inoculated, and also glucose agar slopes, these being grown under anaerobic conditions.

One boxful of tubes was first treated in this manner. The box contained the following materials; all being in test tubes sealed with wax:

1. Mud. 300 fathoms; latitude 66° 55' South, longitude 145° 21' East; ooze, with diatoms; temperature 1.8° C.
2. Mud. 240 fathoms; latitude 66° 52' South, longitude 145° 30' East; ooze.
3. Mud. 230 fathoms; latitude 65° 48' South, longitude 137° 32' East; ooze (no fishes); temperature 1.4° C.
4. Mud. 150 fathoms } latitude 66° 32' South, longitude 141° 39' East; ooze;
5. „ 150 fathoms } temperature 1.62° C.

1. *Mud. 300 fathoms*; latitude 66° 55' South, longitude 145° 21' East; ooze, with diatoms; temperature 1.8° C. The tube contained a greyish, semi-plastic material adhering to its sides.

*Plate A.*—One large and two small, round, creamy, semi-translucent colonies. Stained by Gram's method, both types of colonies showed Gram-negative colon-like bacilli, short and long forms being present in the smaller colonies.

The organisms were tested on agar, serum, potato, gelatine, and the five test "sugars" (glucose, mannite, dulcitol, lactose and saccharose).

*Agar.*—Whitish, creamy, semi-translucent growth.

*Serum.*—Whitish, slimy growth.

*Potato.*—White growth.

*Gelatine.*—Scanty, whitish growth down the needle track, scanty growth on the surface; no liquefaction of the media.

*Sugars.*—No change took place on the first five "sugars."

*Milk.*—No change.

*Plate B.*—One large yellow colony and several creamy colonies. Four cultures were stained by Gram's method.

Yellow colony—a Gram-positive coccoid form (? "yeast").

Creamy growth (two colonies)—a Gram-negative colon-like bacillus, in one case showing short and long forms.

Fine, clear growth—a Gram-negative, curved bacillus or vibrio.

	Agar.	Serum.	Potato.	Gelatine.
"Yeast" (?)	Yellow growth	Raised, yellow growth	Yellow, raised growth.	Scanty growth down the needle-track, spreading on top around the point of inoculation; yellow, slightly wrinkled.
Vibrio	Fine, greyish-white colonies.	No apparent growth...	No change	No change (? subculture died).
Coliform				
Bacilli (1)	White, slimy growth	Small, white colonies...	No change	Nil. (? Alive.)
(2)	White, slimy growth	White, slimy growth...	No change	White growth down the track and spreading out on top.

On the "sugars" the vibrio and the coliform bacilli produced no change. Milk was not affected.

*Subculture 23-3-15.* From subculture dated 20-9-14.

1. A 1. Spreading, semi-transparent, coliform growth. Stained: A narrow, Gram-negative, coliform bacillus, mostly small, but some large serpentine forms.
2. No growth. Later a whitish, slimy growth at the top of the tube, which stained as irregular Gram-positive bacilli, like *Bacillus diphtheriae*, with short, curved forms.
1. B 2. Abundant growth of raised, rounded colonies, lemon yellow in colour. Gram-positive coccus in masses, amongst which were some large forms.
3. No growth. Re-inoculated from another culture of 8-4-15. No growth.
5. No growth. Ditto.
6. No growth. Ditto.

2. *Mud*; 240 fathoms; latitude 66° 52' South, longitude 145° 30' East; ooze.—The tube contained a greyish material adhering to its sides.

*Plate A.*—Several large, whitish grey colonies of a mould. One small, crenated, yellowish colony—a Gram-positive bacillus.

*Plate B.*—Several colonies of a mould and one small, yellowish colony of a Gram-positive bacillus.

The Gram-positive bacilli were tested on serum, potato, and gelatine:—

	Agar.	Serum.	Potato.	Gelatine.
A	White, caramel-like, raised growth.	Moist, whitish pellicle, wrinkled at the edges; digests medium.	White, honeycomb growth, medium dirty yellow.	Large bubble of liquefaction; at the bottom of the bubble a thin, somewhat wrinkled membrane and whitish growth down the needle track.
B	Yellow growth	Pale yellow growth	Yellow growth	Scanty growth down track, and a heaped-up yellow growth on top.

*Subculture 23-3-15.* From subculture dated 20-9-14.

II. A 1. Dense, whitish growth like enamel; Gram-positive bacillus, with long and slender forms, and later showing spores.

II. B 1. Lemon-yellow growth of rounded colonies. A Gram-positive oval coccus, or cocco-bacillus—more like a bacillus—some appear Gram-negative.

3. *Mud*; 230 fathoms; latitude 65° 48' South, longitude 137° 32' East; ooze (no fishes); temperature 1.4° C.

Hard calcareous fragments.

Plated.

Plate A.—No growth.

Plate B.—Five colonies appeared :—

(1) Gram-positive coccus.

(2) Gram-positive "yeast."

(3) Gram-negative bacillus. } Subcultures show the wrinkled, white growth of  
(4) Gram-positive bacillus. } a sporing bacillus.

(5) Long, thin, Gram-negative bacillus, not acid-fast.

### CULTURE REACTIONS.

	Agar.	Serum.	Potato.	Gelatine.	Broth.	Sugar Media.
1	White, slimy growth.	Whitish growth ...	Dirty, greyish brown growth.	Whitish pellicle-like growth down the track and around the puncture.	Turbid. No chains.	.....
2	Pink growth.....	Pink... ..	Two pink colonies.	Scanty growth down the needle track and heaped up on the puncture.	Turbid	.....
3	Greyish white, slimy growth.	Liquefied; reddish tinge.	Raised, slimy, brownish growth.	No change. Subcultures show no growth.	.....	No change.
4	White, slimy growth.	White, filmy growth; digests the medium.	Honeycomb growth; medium dirty yellow.	Liquefied, flocculent growth in the liquid and white pellicle on top.		
5	Yellowish growth.	Yellow, slimy growth	Scanty yellowish, pale growth.	Fine growth becoming narrow down the track; around the puncture yellowish.	.....	No change.

Subculture 23-3-15. From subculture dated 20-9-14.

III. B 2. No growth. Re-inoculated from another culture of 8-4-15 and incubated.  
No growth.

3. No growth. Ditto.

4. Copious, wrinkled, white growth, with irregular edges. Rather short, Gram-negative; sporing bacilli; a number distinctly Gram-positive. Probably the old forms are Gram-negative.



5. No growth. Re-inoculated from another culture of 8-4-15. No growth.

5. (Second tube.) Abundant whitish growth, wrinkled. Moderately long, moderately thick, Gram-positive bacilli, and short, oval, Gram-positive bacilli containing spores. Probably contamination has occurred from neglect.

4. *Mud*; 150 fathoms; latitude 66° 32' South, longitude 141° 39' East; ooze; temperature -1.62° C.—In macroscopic appearance, a greyish material with a slight greenish tinge adhering to the walls of the test tube.

*Plate A.*—(1) Several creamy colonies—a Gram-negative, coliform bacillus.

(2) Several smaller, yellow colonies—Gram-positive cocci.

*Plate B.*—(1) Several creamy colonies—Gram-negative, coliform bacilli.

(2) Several minute, yellow colonies—Gram-positive diphtheroid bacilli.

#### *Cultured Characters.*

	Agar.	Serum.	Potato.	Gelatine.	Broth.	Sugars.
A 1	Whitish, slimy growth, as in B 1.	Translucent, whitish growth.	No change	Scanty growth down the track, spread out on top, translucent and opalescent.	.....	"Acid" in glucose; no change on the other "sugars."
A 2	Abundant, yellow, separate colonies, raised and rounded.	Yellow growth.	Yellow growth.	Slight growth down the needle track, heaped up above and yellow.	Turbid, no chain formation.	.....
B 1	Slimy, whitish growth; in spreading parts, white, semi-translucent and coliform.	Creamy, whitish growth.	Whitish growth.	Fine growth down the track; thin, translucent growth on top but thicker in the centre.	.....	.....
B 2	Yellow ... ..	Golden yellow growth.	Yellow ... ..	Scanty growth down the track and heaped up on top; growth yellow.	.....	.....

5. *Mud*; 150 fathoms; latitude 66° 32' South, longitude 141° 39' East; ooze; temperature -1.62° C. Greenish-grey material, adhering to the sides of the tube.

Plated.

*Plate A.*—Two large, round, greyish colonies of a mould.

*Plate B.*—Two similar colonies.

The cultures were discarded.

## CHAPTER VI.\*

## COMPARATIVE SUMMARY—BACTERIA OF ICE AND SNOW.

THE researches we were able to prosecute in the subject of the 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. One is always liable to pass rapidly from the particular and local truth to the general conclusion, and so the facts as they stand should be marshalled and thoroughly analysed in view of their unusual interest.

Dr. Ekelöf, whose investigations, 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. Rightly he comes to the conclusion, on the evidence of his examinations of soil and on account of the boisterous and 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. But there still remains the chance that some of the bacteria had an aerial origin.

Dr. Gazert, 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, 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 20 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 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 Captain Scott's British Antarctic Expedition (1910-1913) apparently made bacteriological examinations of snow.†

"Atkinson is pretty certain that he has isolated a very motile bacterium in the snow. It is probably air-borne; and, though no bacteria 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."

\* NOTE.—A summary of this chapter has already appeared in "Nature," London, 1918.

† 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.

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 was led, after more extended enquiries, 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 downward in *névé*-fields, declining gradually for hundreds of miles, to fall abruptly in glacial slopes to the sea (Plate-17). In fact we were on the verge of the Continent, with no naked mountains or outcropping nunataks\* encircling us to the south, so far as we were able to judge from sledging journeys into the interior. That is to say, there were in the hinterland no indigenous bacteria of Antarctic soil liable to contaminate the ice and snow, and, as an additional safeguard, so to speak, there was a continuous torrent of air always blowing towards the north. The average hourly velocity of the wind, during our two years' sojourn in Adelie Land, was actually almost 50 miles per hour. The main base (Plate 18) with its few rocks was at sea-level, and behind it mounted the glacier back to the vast, upland plain, which extends southwards, for the most part at a height of 6,000 feet, across the crown of the pole, itself at an altitude of more than 10,000 feet.

It will be seen, from a perusal of the section dealing with cultures of ice and snow, that the results obtained from an examination of frozen algæ and frozen seaweed led us to enquire further into the bacterial content of the glacier ice—apparently as pure as distilled water. In fact, it was through using the thawed ice in various cover-slip preparations that our attention was first directed to the presence of bacteria in unusual numbers, and more systematic observations were then made on the upper slopes of the glacier.

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

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\* The Western Party, under Mr. F. H. Bickerton, discovered a small piece of rock on the snow at a height of 3,000 feet, 17 miles south-west of the Hut in Adelie Land. This was subsequently identified in Melbourne by Professor Skeats and Mr. Stillwell as a meteorite.

Antarctica; algæ, 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. In the winter the ice is discoloured a greyish green tint, while any slimy strands on the surface which are not incorporated with the solid ice become extruded in the air and soon dry into dirty grey tufts. In this friable form they are most suitable as test-tube samples, and numerous specimens were carried back for examination in Australia.

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. A Gram-positive, sporing bacillus spreading as an abundant, pale, wrinkled and adherent growth on all media.
3. Gram-positive, chained 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.

It is probable, from the Log record, that cocco-bacilli and other bacilli are also present; for in Australia it became obvious that the dried specimens of algae contain bacteria, fewer in numbers (and doubtless in species) than appear in the thawed ice locally examined.

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 from 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.

Frozen seaweed, procured as a substratum of fresh water ice, produced Gram-positive cocci and bacilli in smears, and, in culture, a slender, Gram-negative bacillus. This seaweed had been at one time washed up—maybe in a subglacial channel—by the waves, and then had become encased in moving glacier ice. The specimens in question came from the bottom of a glacier shaft of 12 feet, some 40 yards from the sea.

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.

A detailed account is given in the Bacteriological Log of the various precautions which were taken to eliminate fallacies, and of the conditions of environment under which the cultures were made. Thus, nothing more than a bare summary of the results will be necessary as a preface to any inductive conclusions which may be drawn.

1. In a Magnetic Cave, cut shaft-like through the slope of blue ice, about 1,100 yards south of the Hut, at an altitude of 300 feet 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 1,000 yards south of the Hut occurred cocci, motile bacilli, yeast-like bodies, and protozoa.
3. The surface ice at 1,100 yards—altitude 300 feet—yielded in cultures cocci (staphylococci) and short, stout bacilli.
4. At 1 mile—altitude 600 to 700 feet—in surface ice, appeared in culture Gram-positive staphylococci and slender Gram-negative chained bacilli. Protozoa and yeast-like bodies were demonstrated in the thawed ice-chips.
5. Two miles back, at an altitude of 1,000 feet, cultures revealed short, stout, Gram-negative bacilli, Gram-negative cocco-bacilli, Gram-positive staphylococci, and a Gram-positive "yeast." Protozoa were also seen.
6. In the vicinity of Aladdin's Cave, 5 miles south of the Hut, and at an altitude of 1,500 feet, 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 feet 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 cultures.  
In ice at 7 feet—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.
7. At 8 miles, thawed ice contained Gram-positive cocci and slender Gram-negative bacilli. There is no mention of protozoa or yeast-like bodies. Cultures confirmed the presence of the Gram-positive coccus, and produced as well a Gram-negative cocco-bacillus.
8. From the Cathedral Grotto, at 11 miles, and at an altitude of 1,800 feet above the sea, specimens of ice gave in cultures growths of a Gram-positive coccus and a Gram-negative cocco-bacillus. No mention is made of protozoa or yeast-like bodies in the preparations from thawed ice.

9. In a position, 50 miles west of the Hut and 25 miles inland; nearly 4,000 feet 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 nor yeast-like bodies were seen. Many of the bacilli were clumped in zoogloea masses. 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.
10. Lake ice (from the glacial lakes near the Hut) had relatively few micro-organisms in its surface layers; the cocci and bacilli which were seen both tended to aggregate in zoogloea masses. Cultures produced fine growths of a Gram-positive coccus and a Gram-negative cocco-bacillus.
11. In all the cover-slip preparations made from the various samples of ice and snow, elaborate precautions were taken in, first of all, cleaning and then heating over a spirit lamp slides and cover-glasses. Nevertheless, in most instances, minute fibrous particles like slender bacilli, nodular, linear or irregular particles like foreign bodies, and, in some cases, large fibro-cellular strands, possibly of vegetable origin, were seen. Such appearances were neglected, except very occasionally, in the regular entries of the Bacteriological Log.
12. Examinations of glacier ice from various depths in a shaft tended to show that protozoa and yeast-like bodies inhabit the surface layers, and that in this situation free-swimming bacteria are most numerous. No relation could be established between the number of organisms at different depths, owing to contamination of the ice deep down in the shaft by a small amount of soil and grit from the underlying solid earth. Zoogloea masses of bacteria appeared to decrease from below upwards, and were not numerous in the ice at the surface.

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

It was to Dr. J. B. Cleland that we were indebted for a consignment of freshly-prepared culture tubes which arrived by the "Aurora" on her last cruise of relief in the summer of 1913 to 1914. All the tubes reached Adelie Land in good condition, and, to prevent any possible contamination by mould, had been sealed with paraffin.

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 who passed up and down during the summer. In the situation where the cultures were made, the deeper strata of ice were not altogether free of small particles of grit, but to the eye the surface layers appeared clear and blue, and had doubtless passed through the stage of compacted snow and *névé* to glacier ice.

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  $10^{\circ}$  to  $15^{\circ}$  C.; as a general rule the temperature was between  $18^{\circ}$  and  $20^{\circ}$  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 recognised 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, Macfadgen and Rowland have demonstrated that several bacilli will bear exposure for a few days to the temperature of liquid air ( $-192^{\circ}$  C. to  $-183^{\circ}$  C.). More recently it has been proved that certain bacteria actually survive the temperature of liquid hydrogen ( $-252^{\circ}$  C.), applied for as long a period as 10 hours. Bearing in mind such experiments conducted *in vitro*, we could understand that organisms carried by dust-motes to the vicinity of the south geographical pole (at an altitude of approximately 10,000 feet) would probably, if not strictly thermophilic, retain their vitality in a temperature of  $-100^{\circ}$  C. ( $-148^{\circ}$  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 possibly might increase in numbers.

On the other hand, bacteria and their spores have almost a defined limit of resistance to heat— $57^{\circ}$  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 holds good 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, 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, paralysed for long winter months, active and acclimatised 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.

It is instructive to bear in mind the morphological relations of protozoa, algæ fungi and bacteria. Apparently the most modern view is that bacteria are the primitive unicellular organisms which stand as a basic entity of life before their differentiation into algæ on the one hand, and protozoa on the other hand. Again, by stressing vegetable types, certain algæ and the bacteria have been correlated in one class as Schizophyta, with the algæ as schizophyceæ and the bacteria as schizomycetes or splitting fungi. Certainly the fungi were ubiquitous enough in our small sphere of observations. The lichen—a symbiosis of alga and fungus—grew amongst the rocky crevices and on the sun-warmed slabs over every outcrop we saw in Adelie Land and King George V. Land. Fungi, represented by moulds of many species, grew luxuriantly along the rime-filled cracks between the inner lining boards of the Hut, though no indigenous mould was ever seen amongst the soil, guano or rocks. Then, too, many deposits have been found in Antarctica in the form of peat-beds, composed of compact detritus of fungi. Apparently the mere cold, as long as moisture is not wholly frozen, is no deterrent to life and procreation among the Schizophyta. Especially, too, should one take heed of the fact that higher forms of life, represented in Antarctic seas, as coelenterates, crustaceans and fishes, may exist for prolonged periods in a temperature almost 3 degrees below the freezing-point of fresh water, their optimum, annual temperature standing in the vicinity of 0° C. Penguins and seals, with their heat-regulating mechanism and protective blubber, are able to endure intense cold.

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 Murray\* of Sir Ernest Shackleton's British Antarctic Expedition (1907–1909) 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 centre 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. Again they were put into sea-water and into the brine from the bottom of Green Lake, which is so salt that it freezes only at

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\* *The Heart of the Antarctic*, by Sir E. H. Shackleton, C.V.O. Vol. II, p. 238. London. 1909.



about zero (Fahr.). They were left in these salt waters for a month, yet as soon as they were transferred to fresh water they began to crawl about as though nothing had happened.

“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 (a range of more than 200° F.). It is not the eggs merely that survive all these changes, but the grown animals. These are animals comparatively high in the scale. The rotifers are worms, and the water-bears (which stood the same tests) are cousins to the insects and spiders. Some very lowly plants are not killed by being put in boiling water, and doubtless many very simple animals can live through cold greater than we found in the Antarctic.”

It was Murray's opinion that these rotifers and water-bears, on the evidence of the small number of their species discovered in Antarctica, and because of their universal distribution, had been transported from lower latitudes and were not survivors of an era when the southern continent was more or less immune from glaciation.

It would seem that bacteria are 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 is 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, just as the rotifers found under 20 feet of ice in the salt lakes at Cape Royds. Probably many of these bacteria were borne by dust-motes of the air from distant lands, or had thawed out of icebergs broken from the continental glacier, and were capable of taking up a new function as denitrifying organisms. This is merely a suggestion which has never received proof.

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 endeavour 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\*, 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.” A new

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\**Ice and its Natural History*, by J. Y. Buchanan, M.A., F.R.S., M.R.I.

conception at once arises as to the exact definition of the freezing-point of a substance. Buchanan defines it to be "the temperature at which it, as a liquid, passes into itself as a solid; and its melting-point to be the temperature at which it, as a solid, passes into itself as a liquid."

Therefore, we may imagine that when sea-water freezes, the primary solidification which takes place is of the freshwater 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. Actually sea ice is always in motion, and the increased internal pressure due to buckling of the floes causes the concentrated brine (cryohydrates) to be squeezed out in places on the surface where, if the temperature becomes reduced sufficiently, it freezes in the form of crystalline ice-flowers. Again, if we look at the process from the opposite point of view, we note that the greenish blocks of sea ice thrown up in pressure ridges, which are exposed for long to the rays of the summer sun, thaw out first of all throughout the saline meshwork; the salt water draining away by gravity to lower parts of the block, leaving a residuum of fresh-water crystals. The upper part of the mass, when disturbed, collapses in heaps of light-blue gravel, thawing, on being collected, into fresh water.

Buchanan makes the whole matter perfectly clear in the following passage, extending his principle to purer forms of ice, such as glacier ice:—

"All natural waters, including rainwater, contain some foreign and usually saline ingredients. If we take chloride of sodium as the type of such ingredients, and suppose a water to contain a quantity of this salt, equivalent to one part by weight of chlorine in a million parts of water, then we shall have a solution containing 0.0001 per cent. of chlorine, and it would begin to freeze and to deposit pure ice at a temperature of  $-0.0001^{\circ}\text{C}$ .; and it would continue to do so until, say, 999,000 parts of water had been deposited as ice. There would then remain 1,000 parts of residual water, which would retain the salt, and would contain, therefore, 0.1 per cent. of chlorine, and would not freeze until the temperature had fallen to  $-0.1^{\circ}\text{C}$ . This water would then deposit ice at temperatures becoming progressively lower, until when 900 more parts of ice had been deposited, we should have 100 parts residual water, or brine, as it may now be called, containing 1 per cent. of chlorine and remaining liquid at temperatures above  $-1.0^{\circ}\text{C}$ . When 90 more parts of ice had been deposited, we should have 10 parts of concentrated brine containing 10 per cent. of chlorine and remaining liquid as low as  $-13^{\circ}\text{C}$ . In the case imagined we assume the saline contents to consist of Na Cl only, and with further concentration the cryohydrate would no doubt separate out and the mass become really solid. On reversing the operation, that is, warming the ice just formed, we should, when the temperature had risen to about  $-13^{\circ}\text{C}$ ., have 999,990 parts of ice and 10 of brine containing 10 per cent. of chlorine. Now, owing to the remarkable fact that pure ice in contact with a saline solution melts at

a temperature which depends on the nature and the amount of the salt in the solution, and is identical with the temperature at which ice separates from a solution of the same composition on cooling, the brine liquefies more and more ice at progressively rising temperatures, until, as before, when the temperature of the mass has risen to  $-0.1^{\circ}$  C. it consists of 999,000 parts of ice and 1,000 parts of liquid water containing 1 part of chlorine. The remainder of the ice will melt at a temperature gradually rising from  $-0.1^{\circ}$  to  $0.0^{\circ}$  C."

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 super-imposed 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 rainwater of a more temperate climate. And, considering that this contamination by dust-motes has gone on for countless aeons, the whole thickness of the polar ice-cap is impregnated with minute foreign bodies. In the sea, dust-motes—inclusive of impalpable volcanic matter and meteoric material—sink in myriads to the bottom, and form with the minute skeletons of living organisms the wide-spread deposits of radiolarian, globigerina and diatomaceous ooze. The bottom of the sea, however, is more or less stationary, and the deposits are cumulative, whereas the glacier is always in movement, ever increasing in thickness, yet being worn away by a process of ablation in which temperature, humidity, wind velocity and the amount of drifting snow are all modifying factors. Ablation is a phenomenon which primarily affects the snow-waves or sastrugi which cover the many square miles of the southern plateau, so that their form is ever changing. So, too, the structure of the underlying compact snow, of the *névé* and the true glacier ice, are never constant under the stresses and strains of forward movement, super-imposed weight and reaction of the underlying land. In physical terms, the great continental glacier is to be conceived as an immense resilient mass of changing fluidity which crowns the southern extremity of the globe.

Returning to the dissection of a piece of the glacier, we find that a disintegration of the interlocking grains, similar to that which occurs in the 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.

Thus we have come imperceptibly to the conclusion that Antarctic ice, like rainwater, has its impurities, which are derived alike from floating particles in the air as from the combined and uncombined elements of the atmosphere. No form of natural water occurring in the solidified crust of the earth is absolutely pure, and one may say that life is universal over the terrestrial surface within certain limits of depth.

The explanation we have furnished of the anatomical composition of a piece of ice has been purposely elaborated, so that the proof of the manner in which organisms so small as bacteria live and multiply in the ice shall follow more lucidly.

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. Then, too, in an isolated lump of ice, subjected to thawing warmth, the liquid in the upper system of canaliculi would tend to settle down by gravity to the bottom and run away, exuding everywhere from the external pores and drenching the sides of the lump. But the same lump, as an integral part of the glacier, would still be perforated with devious and circuitous passages, 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. The small cracks—part of the infinite system of cleavage lines branching away from crevasses—would form receptacles for this fluid of dissolved salts. The gradient of temperature in ice depends, in a general fashion, on the temperature of the air, which exerts its influence to a certain depth. Below this is a zone where the ice approaches a mean annual temperature.

Sufficient has been said to indicate that if in the section of ice we are considering the temperature approaches close 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  $-13^{\circ}$  C., though in Antarctica, where the ice is purer, it should be four or five degrees higher. Granting that such a temperature may be a few degrees from the actual truth, we may at least be sure that for five degrees 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  $-15^{\circ}$  and  $-20^{\circ}$  C., but on mounting the plateau, which falls steeply to the coast, the temperature descends at the rate of almost four degrees for every 1,000 feet. In the summer time, the shade temperature registered on several occasions  $5.5^{\circ}$  C. ( $40^{\circ}$  F.), and for three

months, at least, the temperature, except for unusual fluctuations due to blizzards, never fell much below  $-10^{\circ}$  C. and was very often close to  $0^{\circ}$  C. Considering, too, that there is a considerable amount of sunshine between the equinoxes, the period during which bacterial life and growth would be possible might be extended, during a favourable 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  $0^{\circ}$  C., at the same time as the thermometer in the sun rises to  $16^{\circ}$  C. In various ice caverns which were dug in the glacier—in two cases at an altitude of more than 1,000 feet—the warmth of the sun, piercing a thickness of six feet of ice, was more than perceptible and sufficient to cause a marked thaw, though the shade temperature might have been below  $0^{\circ}$  C. On such occasions the surface ice and snow became humid and soggy, and over the ice-cliff at sea-level rills of fresh water could be seen trickling into the ocean. Unfortunately we have no exhaustive figures available of the temperature gradient in the glacier ice, under definite atmospheric conditions and at various altitudes.

Still, we may be sure that the thickness of the ice-cap investing the frozen continent in the vicinity of Adelie Land—and the same rule holds good for other Antarctic lands—is not much more than 120 feet, considering that the barrier-like face which the glacier presents towards the sea, for many miles east and west, does not exceed that amount in altitude. A complication arises in the case of the deep glacial valleys, from which immense volumes of ice may debouch into the sea as floating tongues, or as a wide expanse like the Ross Barrier, 500 to 600 feet in thickness. And even at a great distance inland there are probably many deep valleys, though in such cases one would expect in the surface contour of the plateau a corresponding sag, which would tend to fill with accumulations of snow. Neglecting the many speculations which naturally arise, we have one important point at issue, which 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 6,000 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 plenitude 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 south-east 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 cover-slip cocci, motile bacilli and, invariably, zoogloea masses of bacteria in moderate numbers. Single 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 vapour, 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 may come, under the influence of the great poleward-flowing current. The bacteria meanwhile have cooled, become paralysed, and have, 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 snow-flakes, sinking lower with the descending strata of air and alighting at last on the surface of the plateau.

And now, sparse 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 snow-flakes—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 certain 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 decrease 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. (Plate 19.)

We may now quote once more from Buchanan, who refers to the glaciological researches of Hugi:—

“In the Alps the greatest amount of snow falls at a height of from 2,000 to 2,500 metres above the sea. The crystalline snow of the mountains takes the granular form much more easily than does the flaky snow of the lowlands.

“It is to Hugi that we owe most of our exact knowledge and detailed description of the *névé* or firn, of its genesis and of its metamorphoses. He built a hut on the Finsteraarfirn at an elevation of 3,300 metres, and inhabited it for a considerable time for the sole purpose of studying the firn or *névé* and its natural history. He traces the development of the *névé* from the fine crystalline snow of the highest levels, and observes it as it passes into glacier. At a height of 3,000 metres the transformation has taken place at a depth of seven metres below the surface of the *névé*; at an elevation of 2,700 metres it is met with at a depth of a few feet, and at a height of 2,400 metres the *névé* has passed into glacier at the surface. In experimenting on the *névé*, he found that when a hard compact mass of it was exposed to the influence of rising temperature, the binding material of the grains soon dissolved to water without the grains themselves being apparently attacked at all. A lower temperature then reunites the grains so that the whole appears as a uniform compact mass. This shows the lower melting-point of the less pure cementing mass of ice . . . . . All the changes which we witness taking place on the incline between the most elevated *névé* and the lowest extremity of the glacier in the valley, are repeated on the vertical, between the upper and the under surfaces of the *névé* and the glacier. In both directions we observe greater age and more definite development of the mass. Further, what we observe in both these directions we observe also in the individual grain. The older kernel of the *névé* is compact, and blue like the lower glacier, while the white spongy rind on the outside is more of the nature of snow, like the highest *névé*, and passes by layers into the compact central grain. Also in the case of the individual grain, the nucleus or kernel is the first and oldest, and only by continued development does the rind shape itself and gradually pass into the mass of the nucleus and so become a glacier-grain, which then continues its development as the glacier itself continues its own development. In these relations lies the foundation of the whole natural history of the glacier. . . . .”

Here we have a very concise and clear statement of what one would be led to expect in studying a vertical section of the glacier from above downwards; crystals in every stage of development. And, granting that the ice of the Alps is much less pure than the continental ice-sheet of Antarctica, the history of the snow-crystal is still the same; the essential difference residing in the freezing-point of the cementing material. Thus in Antarctica the salts in solution are more diluted; the total freezing taking place at a higher temperature.

One is naturally curious to know how large are the crystalline kernels of snow. Buchanan places their weight at one to two-centigrammes. Compared with the fully developed ice-crystal, whose weight may vary from 100 to 700 grams, it is exceedingly small.

Buchanan, commenting on the size of the grains taken from an old block of ice, says:—"They are particularly interesting when we reflect that every grain, even the largest, has grown, according to the rigid laws of crystallomorphic development, from a single snow-crystal which probably weighed no more than one or two centigrammes."

The last problem we may consider is, what position do bacteria occupy in relation to the crystal? Or again, at what stage in freezing are bacteria deprived of their liquid medium?

Considering that the fresh water content freezes first in a particle of moisture floating in the atmosphere, the first snow-crystal, so to speak, should be perfectly pure. But if we consider how minute are bacteria (a few thousandths of a micro-millimetre), and if we suppose that the first snowy grain weighs one centigramme, it will not be difficult to imagine that bacteria may be included or entangled in the primary solid, though it is more rational to believe that they are added in the peripheral accretions. Still such a question is unimportant as long as proof is available that bacteria may live and under favourable circumstances circulate and increase in numbers within the Antarctic glacier.

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, travelling 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 others are stranded on the littoral, from 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.



## CHAPTER VII.

## ADDITIONAL CULTURES FROM SOILS AND ALGÆ, WITH COMPARATIVE NOTES.

A NUMBER of specimens of morainic mud, granite sand and dried algæ, which were collected in Adelie Land (Plate 16) during the winter of 1913—one in the winter of 1914—were not examined in Sydney until March, 1917, owing to the exigencies of the War and other circumstances. The samples—and there were several of each—had been placed in sterile test tubes sealed with cotton wool plugs; the tubes containing the algæ and one specimen of morainic mud had been closed with cotton wool and sealing wax.

In preparing the cultures, the same procedure was followed in each case. Small fragments of the material to be examined were placed in tubes containing sterile broth. From these, agar was inoculated and plated, agar slopes were cultured, and anaerobic stabs were made in glucose agar, sealed above with a layer of the same medium. One set of cultures was grown at 18° to 20° C., and the other at 37° C.

It was soon found from smears that the bacteria present in the morainic mud and dried algæ were in diminished numbers, compared with the results of our previous examinations in Adelie Land. It was also apparent that the optimum temperature of growth was in the vicinity of 37° C., and not 18° to 20° C., as had been the case in the Hut. Evidently the bacteria had become acclimatised to room-temperature during the three to four years which had elapsed, and preferred to grow most readily at 37° C.

As soon as the colonies appeared on the plates and agar slopes, subcultures were made until the various species were isolated, when their characters were tested on different media.

*Morainic Mud.*

Two samples were available; the first gathered during the winter of 1913, the second during the winter of 1914.

I. The test tube contained bluish grey, rather heavy dust which had been collected at Cape Denison, 150 feet to 200 feet above the sea, at the junction of morainic boulders and glacier ice; many dried clods were mixed with the soil. The cotton wool plug had been burnt off and covered over with sealing wax.

A few particles were placed in sterile broth and the various cultures inoculated.

After seventy-two hours at 37° C., a single, fine, circular, white colony had appeared on the agar plate. This was picked off and subcultured on an agar slope. After a few days, a fine, pale growth had appeared, showing first as minute, almost colourless, circular colonies which fused slowly. Gram-positive cocci of varying size were seen in smears.

II. The test tube, sealed with a plug of cotton wool, contained a bluish grey, heavy dust which was perfectly dry:

A solution of the soil in broth was made, more concentrated than in the case of I, as bacteria were few in smears.

After seventy-two hours at 37° C., the agar plate exhibited twenty-three colonies in all; of which twelve were small, circular, white growths, three were yellow, circular colonies, one was a rose pink, circular colony, two small growths (possibly a contamination) were whitish moulds, and of the rest, a few were fine, light yellow in tint and circular in shape, while the rest were greyish and wrinkled. Subcultures were made of the rose pink, yellowish and white colonies, and of the greyish, wrinkled growth. The moulds were neglected.

As a result of subcultures from I and II, the following six organisms were isolated, each of which will be followed in its more detailed reactions:—

- (1) A Gram-positive sporing bacillus. (Plate 10, fig. 1.)
- (2) A Gram-positive coccus. (Plate 10, fig. 2.)
- (3) A Gram-positive cocco-bacillus. (Plate 10, fig. 3.)
- (4) A Gram-negative bacillus. (Plate 10, fig. 4.)
- (5) A Gram-positive chained bacillus, sporing. (Plate 11, fig. 5.)
- (6) A Gram-positive coccus. (Plate 11, fig. 6.)

(1) Fairly stout, actively motile, bacillary rods which vary markedly in length (from 1.7 $\mu$  to 5.6 $\mu$ ) and slightly in width (5 $\mu$  to 7 $\mu$ ), staining readily by Gram's method in cultures 24 to 36 hours old. After that period the bacilli are decolourised, while sporing forms are all Gram-positive. Some of the shortest forms are rather stout and oval. The arrangement of the bacilli is often in packets, closely approximated side by side, though usually they are single, or in twos, end to end.

In culture this Gram-positive sporing bacillus is almost always profuse in growth.

On an *agar slope*, within twenty-four hours, the medium is soon covered with a dense, spreading growth, milky white in lustre, with characteristic processes like villi along the border. These growing processes, also apparent on *serum agar* and on *glucose agar*, project in sharp salients, while minute, circular, white, outlying colonies appear round the base of the villi. In *glucose stabs*, after five days, the medium assumes a brownish colouration.

*Gelatine* is slowly liquefied; after forty-eight hours there is a very fine, white growth along the stab and on the surface; and after three to four days, the gelatine becomes liquefied in moderate amount in its upper part.

On *coagulated blood serum* there develops in twenty-four hours a pale smear, yellowish in tinge, liquefying the medium so that the smear seems to be embedded in it. The liquefaction progresses, so that at the end of seventy-two hours the medium is almost wholly disorganised.

*Broth* in twenty-four hours becomes opalescent, and later is found to be full of flocculi of white growth.

On *potato* a filmy white smear is seen after forty-eight hours; which in four days is replaced by a brownish stain invading the substance of the medium.

In *litmus milk* the bluish colour is observed to be discharging itself after twenty-four hours, but it is from four to five days before the colour has disappeared completely, the milk has clotted and a faint acid reaction has developed.

This Gram-positive sporing bacillus is a facultative anærobe, and is non-pathogenic to guinea-pigs.

(2) Large, non-motile cocci,  $1.1\mu$  to  $1.7\mu$  in diameter, occurring singly, in short chains and in masses, and staining by Gram's method.

On *agar slopes* this organism always appears as a fine, rose pink growth, raised so as to have the contour of a long liquid drop, and well-defined along its slowly spreading border. The growth is rather viscid to the point of a platinum needle, so that small threads adhere to it. On *serum agar* the rose pink colour is more marked, but the growth is still fine and increases very slowly.

In *gelatine*, after four or five days, a fine, rose-coloured, somewhat depressed folium of growth caps the stab, along which is just visible a serrated, pale fringe. There is no liberation of pigment nor any liquefaction in the medium.

After twenty-four hours, on *coagulated blood serum*, one may observe a fine, pinkish, raised and drop-like colony. Later, more drops of growth project along the stroke, and these very slowly fuse into a fine, pinkish smear. No growth is produced in *broth*— but on *potato*, after a few days, a very fine, pinkish growth may be seen. In *litmus milk* no change occurs and the reaction remains alkaline.

This Gram-positive coccus is a facultative anærobe, and is non-pathogenic to guinea-pigs.

(3) Motile cocco-bacilli,  $1.6\mu$  in length and  $1.1\mu$  in width, staining readily by Gram's method. In general, the bacteria are oval, and their arrangement is single, in twos (when they are very similar to diplococci), in short chains or in clumps. Swollen forms, like large cocci, occur, and there are definite bacilli, about  $1.8\mu$  in length.

On an *agar slope*, after twenty-four to forty-eight hours, the growth is always fine and raised; at first egg-yellow in colour, changing to orange, while in older cultures and on *serum agar*, it is bright cerise in tint, raised and thick like a drop of paint, and sharply contoured along its margin.

A characteristic nail-shaped growth follows a stab in *gelatine*; the head of the nail being heaped-up and bright cerise in colour; no liquefaction of the medium.

After twenty-four hours, on *coagulated blood serum*, a very fine, raised, drop-like, orange growth appears, which makes very slow headway. A few threads are produced

in *broth*; the medium being unsuitable for growth. The same holds good of *potato*, on which no colonies are evident. In *litmus milk* there is no change and the reaction is alkaline; though in one instance some clotting occurred after seven days, the reaction remaining neutral.

This Gram-positive cocco-bacillus is a facultative anaerobe, and is non-pathogenic to guinea-pigs.

(4) Bacilli, tending to be slightly curved, actively motile and from  $1.7\mu$  to  $3.4\mu$  in length and  $.4\mu$  in width, decolourised on being stained by Gram's method. The arrangement of the organisms occurs singly and in chains of two, three or more. When, as often happens, they are in pairs, end to end, the bacilli becomes slightly curved and bent towards one another at an obtuse angle. The chains are occasionally very long and extend in serpentine curves almost across the microscopic field. The staining is not always uniform; some of the bacteria being blotchy or with granular markings alternating with clear spaces. This characteristic is more obvious in older cultures, when the protoplasm is gathered up in stained droplets, creating in the case of some bacilli almost a diphtheroid appearance. Involution forms are swollen and elliptical.

On an *agar slope* appears, in twenty-four to forty-eight hours, a transparent, thin smear, which spreads in miniature terraces with a lobulated fringe-like border. Discrete, fine colonies are colourless and circular. When the culture becomes older, the separate, circular colonies become dry and whitish, their form is ovoid, and they adhere to the surface of the medium. The colourless smear dries in stellar clusters. On *glucose agar* the growth is paler in tint.

In *gelatine* one finds an expanded cup filled with a thin, fine smear, tinged a greyish blue; in the medium, along the track of the stab, are pale, circular and lenticular colonies; no liquefaction.

After twenty-four hours, on *coagulated blood serum*, a very fine, pale, slightly raised, linear growth, with a few outlying, minute, circular colonies, is visible. After seventy-two hours, this growth has scarcely extended, acquiring a yellowish tinge. *Broth* becomes turbid in twenty-four hours, and an abundant, white, flocculent deposit is found in the bottom of the tube. On *potato* are seen irregular, creamy markings which fuse, after forty-eight hours, into a pale smear. When *litmus milk* is inoculated, the colour is completely discharged, and a deposit of clot forms; the reaction of the medium remaining faintly acid.

This Gram-negative bacillus is a facultative anaerobe, and is non-pathogenic to guinea-pigs.

(5) Thick, non-motile, Gram-positive, sporing bacilli, on the average  $3.5\mu$  long and  $.8\mu$  wide, occurring in long chains, which persist in growths up to 48 hours old, when the chains tend to become dispersed and involuted forms begin to appear. In a culture, 24 to 48 hours old, bacilli may vary in length from  $1.8\mu$  to  $5.9\mu$ , and some are as thick as  $1.5\mu$ . The involution forms are very curious; the chains break at intervals,

the separate bacilli swell up in almost circular, or boat-shaped bodies, or curl on themselves in chainettes to make scrolls and masses. (Plate 13, fig. 14.) After forty-eight hours, sporing occurs.

On an *agar slope* a profuse and dense, white growth, with a fluffy border, soon appears and may cover the medium almost completely in twenty-four hours. On *glucose agar*, a pale, glistening, uniform smear soon forms on the surface.

There is marked liquefaction of *gelatine* after seventy-two hours. At first one sees a white line along the course of the stab, and above this a white pellicle which has sunk into a liquid cup. Later, the whole medium melts and is filled with filmy masses of floating growth.

A pale, raised growth is produced on *coagulated blood serum*, after twenty-four hours. By seventy-two hours, it is sinking into and liquefying the medium, imparting to the serum a brownish colouration. *Broth* is seen to be turbid in twenty-four hours; and later is filled with shreds and ribbons of white growth. *Potato* is covered with a growth, greyish white after forty-eight hours, but in four days, brownish, imparting the same colour strongly to the solid medium. In *litmus milk*, after twenty-four hours, the colour is completely discharged and small lumps of clot have separated out; the reaction remains neutral.

This Gram-positive, chained bacillus is a facultative anaerobe, and is non-pathogenic to guinea-pigs.

(6) Large, non-motile cocci,  $1.1\mu$  to  $1.5\mu$  in diameter, staining by Gram's method. The cocci, in smears, are of varying size, being arranged singly, as diplococci, or in masses; in old cultures they become very large and swollen.

On *agar*, after a few days, a scanty, whitish, wrinkled and heaped-up growth is seen. On *serum agar* there is a slightly wrinkled, dull-yellow smear with a denticulated edge. Many pale, fine, circular colonies are produced on *glucose agar*.

No growth occurs along the stab in *gelatine*, but is marked on the surface, producing liquefaction. Later, the growth in the liquefied *gelatine* has a yellowish tinge.

On *coagulated blood serum*, after twenty-four hours, fine, pale droplets are observed, which resolve in seventy-two hours into a fine, linear, yellowish ribbon of growth. The cocci make no progress in *broth* nor on *potato*. In *litmus milk* no clotting occurs, and the reaction remains neutral.

This Gram-positive coccus is an aërobe, and is non-pathogenic to guinea-pigs.

#### *Granite Sand.*

Two samples of granite sand, gathered in Adelie Land during the winter of 1913, were available for examination. The surrounding rocks are of gneissic granite and this soil, which is exceedingly scanty, was taken from a crevice.

I. The test tube contained coarse, greyish or dirty-orange grit, admixed with some fine sand; no signs of macroscopic organic matter.

After twenty-four hours at 37° C., an agar plate showed two fine, circular white colonies which were subcultured. Five days later, four minute, pale colonies were evident on the plate. Further subcultures were made.

II. The material contained in the test tube was similar to the previous sample.

After three days at 37° C., no growth had appeared; and after twenty-one days the result was negative.

Two organisms were isolated from I, and the reactions of these follow in detail:—

(7) A Gram-positive coccus. (Plate 11, fig. 7.)

(8) A Gram-positive coccus. (Plate 11, fig. 8.)

(7) Non-motile cocci,  $5\mu$  to  $6\mu$  in diameter, which are Gram-positive, but with a tendency to become quickly decolourised. They occur singly, as diplococci, and in staphylococcal bunches.

In the original plated agar culture, the colonies were fine, pale and circular, but in subcultures on *agar* or *serum agar slopes* the colour became yellowish and the growth was still fine. Later still, the yellowish tinge changed to a canary-yellow or lemon-yellow tint. Finally, the growth was scanty, raised and lemon-yellow, presenting an irregular surface and a lobulated border; with a few, outlying, fine, yellowish colonies. *Glucose agar* seemed a more suitable medium, for, on a slope, there was soon evident a moderate growth of lemon-yellow, circular colonies fusing into a uniform smear.

In *gelatine* a lemon-yellow folium develops on the surface, and fine rows of colonies follow the course of the stab. The folium becomes depressed, sinking, with a slow liquefaction of the medium.

After twenty-four hours, a lemon-yellow drop is visible on *coagulated blood serum*; the growth remaining fine and extending very slowly. A few threads are seen in *broth*, and on *potato* fine islets of canary-yellow growth appear. *Litmus milk* is unaffected, except that the reaction remains neutral.

This Gram-positive coccus is a facultative anaerobe, and is non-pathogenic to guinea-pigs.

(8) Non-motile cocci,  $7\mu$  to  $8\mu$  in diameter, Gram-positive, but decolourised rather easily. The bacteria are arranged either singly or in masses. In old cultures there are many large and swollen cocci.

Growth on *agar slopes* is in the form of a fine, raised, yellow smear with a few outlying, circular colonies. On *glucose agar* the colonies are paler, and growth is more extensive.

*Gelatine* is more quickly liquefied than in the case of (7); the upper one quarter of an inch or more of the medium melting. Yellow growth clouds the liquid and caps the solid medium, but no colonies are seen along the stab.

White, fine, glistening drops are the only sign on *coagulated blood serum*. In *broth* a few threads may be whisked up from the bottom; while, on *potato*, a light yellow smear spreads slowly. No change occurs in *litmus milk*, beyond a resultant neutral reaction.

This Gram-positive coccus is a facultative anaerobe and is non-pathogenic to guinea-pigs.

#### *Dried Algae.*

Two specimens of dried algae were available; both collected during the winter of 1914.

I. The test tube contained greyish green or slaty grey, dry shreds, particles, and wafers.

After three days at 37° C., an agar plate showed a pale growth, having a very faint pinkish tinge, surrounding a small piece of alga embedded in the medium. Subcultures were made. After six days, the growth had reached the surface of the agar, and was spreading on it as a greyish white, wrinkled smear. A small mould had appeared, as well as a single, minute, pale colony, on another part of the medium. The mould was neglected and more subcultures were made.

II. The contents of the test tube were similar to the preceding specimen.

After twenty-four hours at 37° C., two colonies were visible on an agar plate. Twenty-four hours later, nine colonies could be counted; including white, pale yellow, and colourless growths. Subcultures were made.

Four organisms were isolated from I and II, and these will be followed in their detailed reactions:—

(9) A Gram-positive coccus. (Plate 12, fig. 9.)

(10) A Gram-positive bacillus. (Plate 12, fig. 10.)

(11) A Gram-positive, sporing bacillus. (Plate 12, fig. 11.)

(12) A Gram-positive, chained bacillus, sporing. (Plate 12, fig. 12.)

(9) Large, non-motile cocci, of varying size ( $1.2\mu$  to  $1.7\mu$  in diameter), staining by Gram's method, and occurring singly, as diplococci and in staphylococcal masses. Many swollen forms appear in old cultures.

The cocci develop first, as a pale growth, and ultimately, as a fine, yellow, flat smear on *agar*; separate colonies are yellow and circular. On *glucose agar* the growth is very fine and pale.

In *gelatine* is seen a pale cup of growth, with a yellowish tinge, which has sunk with slight liquefaction into the medium; a fine, white fringe following the course of the stab.

On *coagulated blood serum* is produced a yellowish, fine growth which became depressed as a narrow ribbon along the course of a stroke. A few stringy shreds are visible in *broth* after forty-eight hours. *Potato* shows a small, yellow patch changing to a lemon-yellow tint. Slight clotting occurs in *litmus milk*, with an alkaline reaction.

This Gram-positive coccus is a facultative anaërobe and is non-pathogenic to guinea-pigs.

(10) Slender, motile bacilli,  $1.7\mu$  in length and  $.3\mu$  in width, staining by Gram's method. Mostly they are arranged singly, and rarely in twos, end to end, at an obtuse angle.

A glistening, milky white, spreading smear, which in a few days becomes yellowish in tint along a midrib, is seen on *agar slopes*. Eventually the yellow colouration extends almost to the limits of the brilliant white smear, which tends to be raised along its margin. On *glucose agar* the bacilli grow in a greyish white, fairly profuse, raised growth.

Fine, lenticular or circular, white colonies may be observed along the course of a stab in *gelatine*; no growth is visible on the surface, and there is no liquefaction of the medium.

After seventy-two hours, on *coagulated blood serum*, there are only a few, very minute, pale colonies, some of which subsequently take on a yellowish shade. *Broth* becomes opalescent and milky with growth, and on *potato*, after forty-eight hours, is seen a fine, brownish streak. The colour of *litmus milk* is discharged, a clot forms, and the reaction remains acid.

This Gram-positive bacillus is a facultative anaërobe, and is non-pathogenic to guinea-pigs.

(11) Motile bacilli, of variable length ( $1.8\mu$  to  $3.9\mu$ ), and from  $.4\mu$  to  $.6\mu$  in width; the early vegetative forms staining by Gram's method. In twenty-four hours, Gram-negative, sporing bacilli and many non-sporing, Gram-negative forms are seen in smears from cultures. The general arrangement of the bacteria is in packets or single, often in twos, end to end, at an obtuse angle, and very occasionally in short chains.

The cultures of the bacilli are very characteristic; first of all there is on *agar* an opaque, quickly spreading, adherent, raised growth which soon becomes wrinkled and whitish, covering the whole surface of the medium in twenty-four to twenty-eight hours. In older cultures the centre becomes dry and more pitted, so as to resemble honeycomb in colour and appearance; the growing border is irregular. On *glucose agar* a profuse, white, raised and somewhat glistening, adherent growth with lobulated edges appears in twenty-four hours. Wrinkling soon takes place.

On the surface of *gelatine* an irregular, whitish, wrinkled growth is speedily heaped up, and along the stab is a fine fringe; no liquefaction.

Along a stroke on *coagulated blood serum* a well-marked, brownish, wrinkled, raised growth is established in twenty-four hours, and this tends to sink, with slight liquefaction, into the medium. In twenty-four hours, *broth* is turbid and filled with loose pieces of white growth; in forty-eight hours, a puckered, white film covers the surface of the liquid. On *potato* there are produced wrinkled, whitish markings which soon fuse; the medium changing to a light brown colour. After four days, the whole



of the potato assumes a deep brown tint; the dark surface of the growth being mottled and pitted. A dirty yellow clot is formed in *litmus milk*; the reaction remaining faintly acid.

This Gram-positive, sporing bacillus is a facultative anaerobe, and is non-pathogenic to guinea-pigs.

(12) Thick, chained, non-motile bacilli, showing great variability in length ( $1.8\mu$  to  $6.8\mu$ )—on the average  $3.5\mu$ ; and  $.6\mu$  to  $1.5\mu$  in width; on the average  $.8\mu$ —staining by Gram's method. The chained arrangement holds in cultures up to forty-eight hours old; when involution occurs, the separate bacilli tend to swell and curl up, the chains are broken, and circular, scroll-like or arc-shaped bodies are seen. After forty-eight hours, sporing commences.

On an *agar slope* a profuse and dense white growth, with a fluffy border, soon appears and may cover the medium almost completely in twenty-four hours. On *glucose agar*, a pale, glistening, uniform smear soon spreads over the surface.

Marked liquefaction occurs after forty-eight hours in a *gelatine stab*. The upper inch of the medium may dissolve and gauzy flocculi of growth are observed in it.

A pale, raised growth is visible on *coagulated blood serum*, after twenty-four hours. By seventy-two hours, it tends to sink into and liquefy the medium, imparting to it a brown colouration. *Broth* is turbid, within twenty-four hours, and filled with flocculent growth. *Potato*, in forty-eight hours, is covered with an abundant greyish-white smear, and in three or four days the whole of the medium is discoloured deep brown. Clotting occurs in *litmus milk*, the colour is completely discharged, and the reaction remains neutral.

This Gram-positive, chained, sporing bacillus is a facultative anaerobe, and is non-pathogenic to guinea-pigs.

The reactions on all media of (5) and (12) are essentially the same, so that these bacteria are doubtless identical. Chained bacilli were often found in frozen algae (see Chapter I), in Adelie Land.

#### COMPARATIVE NOTES.

When, during 1903 and 1904, Dr. Erik Ekelöf was carrying out his exhaustive researches on the soil of Snow Hill Island, illuminating pages were being added to the subject of Antarctic bacteriology. This island, belonging as it does to the complex of ice-covered land lying to the south of Cape Horn, is a field where outcrops of earth, typical of the neighbouring Graham Land and of the zone of Antarctica, presented themselves to a bacteriologist armed with an incubator, media, stains and the other strictly necessary articles of equipment. Dr. Ekelöf's results are highly important; as distinctive as the contemporaneous evidence of Dr. Gazert on the bacteriology of sea-water.

Dr. Ekelöf started out with a modest expectation, and the richness of his findings must have been astonishing to himself as to those who, previous to the

commencement of the Swedish Antarctic Expedition (1901-1903), gave him scant encouragement in his scientific endeavours. Perhaps, merely for the sake of the valuable work he was able to do during two southern winters, it was fortunate that the Expedition was not relieved in 1902 by its ship, the "Antarctic," which was lost in the ice. No lives were lost through this fatality, but the programme of bacteriological investigations of sea-water, planned by Dr. Ekelöf, was not carried out, and he was able to concentrate his attentions on the bacteria of soil—an ample sphere, judging by the detailed records.

He states, as a preface, his individual attitude to the subject:—

"A very simple consideration in regard to the occurrence of not only many animals in the soil of polar lands, but also of a great number of plants, high in the scale of living, and sedulously investigated by different explorers in the past, should have given rise naturally to the conjecture that in an environment to which such a well-developed life of animals and plants could have adapted itself, it should not be regarded as completely impossible that bacteria, these uncomplicated and primitive organisms, could find the conditions for their existence."

On this just assumption, it is not surprising that Dr. Ekelöf should prove "that in this soil, apparently bare of all life, there survives still a persevering and not inconsiderable flora of bacteria; and that Antarctic soil, during the warmest period of the year, attains a proportion in bacteria which is not much inferior to that of the soil in our own country (Sweden)."

It is instructive to summarise briefly his results, which hold for Snow Hill Island, and doubtless for the patches of bare land within and beyond the American Quadrant of Antarctica:—

- (1) Of 105 samples of soil examined, 11·5 per cent. were sterile and 88·5 per cent. contained bacteria.
- (2) The largest number of organisms per cubic centimetre, demonstrated in a single specimen (collected during the summer of 1902) was 140,000; the average for all samples being 19,000 per cubic centimetre.
- (3) Seasonal variations in the number of bacteria were very marked. For example, the average number of bacteria in the summer was ten times that found in the winter. The heat of the sun (insolation) proved to be a most important factor in promoting growth.
- (4) Bacteria were not present in earth obtained from depths greater than 20 centimetres.
- (5) Gelatine media were largely used and, in incubating soil, it was often six to eight days before colonies could be observed; the optimum temperature of growth being about 17·5° C.
- (6) Twenty-nine different species of bacteria were isolated from the soil; 17 cocci, 11 bacilli (including some spirilla and thread-like organisms), and one

bacterium of an unknown group. The reactions of each species were described in great detail, in gelatine-plate, gelatine-stab, glycerine agar and bouillon cultures.

Dr. Gazert, working at the same time close to the eastern limit of the African Quadrant of Antarctica, made the important discovery of an anaerobic, sporing bacillus similar to *Bacillus tetani* in the guano and moss soil of Gaussberg.

The above results of our work on the morainic mud and granite sand of Adelie Land, in the Australian Quadrant of Antarctica, should be considered in conjunction with the cultures from moss soil and lichen soil gathered in the same locality and described in Chapter IV. The last-named cultures contained Gram-positive "yeasts" and Gram-positive, sporing bacilli. The Gram-positive, sporing bacillus, according to the characters noted by Dr. J. B. Cleland, is probably identical with No. 11.

Looking through the reactions of Dr. Ekelöf's twenty-nine organisms, we were able to find one which corresponds in his partial description exactly to No. 2—a Gram-positive coccus of variable size, with a fine, rose-coloured growth on solid media, not liquefying gelatine and multiplying slowly in broth. Dr. Ekelöf states that the coccus was met with frequently in samples of earth—in some cases these cocci were very numerous—examined at Snow Hill Island. "Yeasts" were found at Snow Hill Island in the form of Gram-positive, branching mycelia; and of these, three species of *Actinomyces* were isolated, and an organism (mould) which the discoverer could not classify.

A few of Dr. Ekelöf's bacilli have appearances (size and arrangement), staining reactions and growth on a single medium similar to ones above described, but they vary in other particulars. Mlle. Tsiklinsky found five organisms (bacilli) in specimens of Antarctic soil—morainic mud probably contaminated by penguins and other birds—brought from the south by Dr. Charcot. In no instance do the characters of any one of these correspond with the bacilli of morainic material gathered in Adelie Land.

Besides the bacilli, Mlle. Tsiklinsky isolated a *Streptothrix*, a species of "red yeast" (Gram-positive, oval cells forming a mycelium), and a Gram-positive coccus. Three kinds of moulds grew from all the specimens of soil. As a result of her observations, she concludes that the bacteria of the South have no special Polar character, and that, although unable to identify with certainty any of the bacteria of Antarctic soil, she is able definitely to state that none of the organisms may be placed in a group peculiar to itself, being rather a variety of species already known and studied.

Finally, one should realise that by reference to the existing classification of bacteria, it is impossible in most cases to do more than describe the reactions of an Bacteriology as a science is more or less systematised. The results of these Expeditions have shown that the bacteria of Antarctic soil are prolific in number, and that a rich field of inquiry and speculation lies open to scientists of the future.

## CHAPTER VIII.

## COMPARATIVE SUMMARY—BACTERIA OF THE SEA AND OF MARINE MUD.

IN this chapter it will be convenient to make brief mention of the work done by other observers on the bacteriology of sea-water, first of all, in connection with the subject of nitrifying and denitrifying organisms. Both Dr. Gazert and Dr. Ekelöf made quantitative determinations, as also did Dr. Harvey Pirie; in each case, with positive results. Dr. Harvey Pirie quotes the interesting conclusion of Brandt; namely, that "denitrifying organisms play an important role in marine metabolism, setting free again the great mass of nitrogen which is brought into the ocean in the form of nitrate, nitrite and ammonia salts, and breaking down dead organic matter. He has propounded the view, based on the fact that polar seas are very rich in plankton, while tropical seas are comparatively poor, that the activity of denitrifying organisms is far greater in warm seas than in cold, while nitrification, on the other hand, is probably more active in polar seas."

He finds this contention to be mainly true, though Dr. Gazert could produce no evidence of nitrifying organisms, and he himself had such slender results that he admits "that nitrifying organisms are not present in these waters, or that the media employed were not suitable for their growth." On the other hand, Dr. Harvey Pirie found that denitrifying organisms of slight activity, under the frigid conditions, were widespread over the Weddell Sea, and Dr. Gazert records a similar generalisation in the area where the *Gauss* worked.

Mlle. Tsiklinsky, from twenty-five cultures of sea-water (presumably the surface-water) made by Dr. Charcot, isolated five species of bacteria and two species of "yeast"; similar forms to some described by Dr. Harvey Pirie. The last-named observer, from a specimen of bottom-water at 2,550 fathoms, grew a short, motile *bacillus*, exhibiting chain formation. Dr. Ekelöf, in a more limited sphere, obtained positive results in his examinations of sea-water.

Dr. Gazert's records were more exhaustive, embracing, as well as those made in Antarctic seas, a large number of cultures secured from bottom-water (uncontaminated by ooze), ooze-water (contaminated by ooze) and ooze in the South Atlantic and South Indian Oceans. He found that the temperature at great depths, passing from the tropics southwards, varied from 2.8° to 0.3° C.

Twenty-four investigations altogether were made of bottom-water, and in only three instances was there a complete absence of bacteria. At a depth of 1,440 fathoms in Antarctica (latitude 65° 28' South, longitude 86° 10' East) twelve organisms per cubic centimetre were present in bottom-water.

Of the total observations (seventeen in all) made on ooze-water, a complete absence of bacteria could only be proved in one case. In Antarctica, five examinations were positive; thirty-two organisms per cubic centimetre being present at 1,440 fathoms.

Among twenty-four samples of ooze, there were fourteen in which no growth could be obtained. In Antarctica, examinations on the seven occasions recorded, in depths down to 2,170 fathoms, gave negative results. Dr. Gazert was, therefore, led to conclude that the oozes of the frozen sea are apparently sterile. He quotes for comparison the fact that B. Fischer did not find any germs in two specimens of ooze taken in the Atlantic Ocean, in the region of the Gulf Stream, in depths of 830 and 1,315 fathoms; and in two other samples, one from the sargasso sea in 2,870 fathoms and another from the north equatorial stream in 2,240 fathoms, he ascertained the presence of bacteria in small pieces of ooze (the size of a pea), but considered that his findings were open to fallacy.

Dr. Gazert says:—

“The results of the investigations in the antarctic regions and in the tropics partake of the same character; in effect, that the deep sea in either case is poor in germs. On account of this fact, and considering that the bacteria in either extreme of latitude have an optimum temperature of growth of 20° C., we may at once come to the conclusion that the sudden change from the icy cold of the deep water to the temperature of the laboratory does not kill the germs; and further, that the observed lack of bacteria is not merely apparent, but existent.”

It is rather remarkable that Dr. Gazert's samples of ooze were in every case but one (diatomaceous ooze) from the glacial clay which one finds covering the sea bottom in places where glacier tongues push off from the land or large masses of ice break away and deposit their burden of continental soil. He concludes his researches with a discussion on the amount of organic matter found in the oozes. In the Antarctic regions, organic substances varied between 2.7 and 4.7 per cent., using the ordinary chemical tests.

One may profitably compare these results with the conclusions reached by Dr. Portier\*, who conducted his researches in the North Atlantic Ocean and the Mediterranean Sea.

“Near coasts, and especially off the outlets of rivers, the numbers of bacteria (in the sea-water) is very large (several hundreds or several thousands per cubic centimetre).

“As one gradually passes to the high seas, this number diminishes rapidly. On the whole, in mid-ocean, so to speak, they are fairly abundant near the surface (several organisms, sometimes ten to thirty per cubic centimetre). But their number diminishes very quickly as one descends into deep water, until, at about 1,000 metres (550 fathoms), one is not able to discover a single bacterium in 30 cubic centimetres of water. The water is perfectly sterile, as pure as spring water.”

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\* Bulletin du Musée Océanographique de Monaco (No. 56). Considérations sur la biologie marine par S.A.S. Le Prince de Monaco. 1905.

\* 20218—N VOL. VII, PART 3.

An exception was found to this rule in the case of a submarine ridge—the Gorringer bank—in the Atlantic Ocean between the Azores and Portugal, where the bottom rises to within several hundred metres of the surface. Here a rich fauna and much decomposing organic matter were found to alter correspondingly the bacterial content of the supernatant water.

On the analogy of this evidence, one would expect in Antarctica to find the bacterial content of the sea greater near outcrops of the coast, where thaw-water in the summer time washes down organisms from the soil, and where seals, penguins and birds abound. Dr. Ekelöf, in the vicinity of Snow Hill Island, discovered that the soil was rich in bacterial life, and from twenty-seven samples of sea-water taken in a locality not far from Graham Land grew cultures in all but six; there being on the average, to every cubic centimetre of water, twenty-one micro-organisms. He was not able to make comparative records in the open sea because of the loss of his ship, the “Antarctic.” Dr. Gazert’s observations yielded similar results off the coast of Kaiser Wilhelm II Land. Dr. Harvey Pirie, working on the “Scotia” in the open Weddell Sea—an area which is mostly beset with pack-ice—found in one anomalous instance 112 colonies in one cubic centimetre of water. This should not be surprising as the local bacteria may have been in this instance increased by a berg with massive inclusions of soil, above or below the surface; material which is continually being carried northwards from the continent by floating ice. Or again, in open leads, amongst the drifting floe, surface plankton in any quantity must entangle many bacteria. It is true that we have no facts to support this suggestion, nor has any extended work yet been done to establish the relation between the number of bacteria in the various zones between the inshore waters and the ocean beyond the fringe of Antarctic pack-ice; but the results we have obtained from investigations of the soil, glacier ice, and falling snow, have been so striking, that new light has been thrown on, at least, one source of the low life prevalent in the Antarctic and Southern Oceans. Dr. Ekelöf concluded that his findings were of great theoretical interest, in that they conclusively proved in the sea-water the existence of bacteria, which were not only able to grow, but also to increase in numbers at a temperature of  $1^{\circ}$  or  $2^{\circ}$  C. Our own experiments tend to show that a similar phenomenon may obtain in the case of the ice and snow which ensheath the great southern continent of 5,000,000 square miles.

During two cruises of the “Aurora,” we were not equipped with the necessary apparatus and media for making a bacteriological examination of sea-water. There is a short record in the Bacteriological Log of a specimen of sea-ice from Commonwealth Bay which yielded several cultures containing (a) short, nodular bacilli; (b) short coccobacilli; and (c) yeast torulæ. Close to the shore—the haunt of seals, penguins and birds for six months of the year—one would expect to meet bacteria in numbers. Phosphorescent patches of water were often noted at night in the interstices between the pancake ice at Cape Denison, and Mr. Hunter, the biologist, found in samples of the water small copepod crustaceans. No examinations were made in search of phosphorescent bacilli.

The observations made in Sydney on four specimens of marine mud are, however, of some interest. In one case where the mud was an example of diatomaceous ooze, from a depth of 300 fathoms, obtained about 6 miles off the coast of Adelie Land, bacteria were plentiful; (a) a Gram-positive coccus; (b) a Gram-negative, colon-like bacillus; (c) a Gram-negative, curved bacillus or vibrio; (d) an irregular, Gram-positive bacillus like *Bacillus diphtheriae*; (e) a Gram-positive "yeast." In the same situation a rich catch was made in the deep-sea trawl of ascidians, crinoids, holothurians, and fish. There is the chance, however, that the specimen of ooze may have been contaminated by the sea-water, as it was collected in a sterile tube as soon as the sounding instrument had reached the surface. Still, other samples gave consistent positive results.

For example, at a station a little further north and further to the west (latitude  $66^{\circ} 52'$  South, longitude  $145^{\circ} 30'$  East), a Gram-positive, sporing bacillus, and a Gram-positive cocco-bacillus, were grown from ooze at 240 fathoms, some 8 to 10 miles off the land. Calcareous fragments recovered in 230 fathoms, 60 to 80 miles north of the continent (latitude  $65^{\circ} 48'$  South, longitude  $137^{\circ} 32'$  East), showed (a) a Gram-positive coccus; (b) a Gram-positive "yeast"; (c) and (d) Gram-positive, sporing bacilli, of two species; and (e) a long, thin, Gram-negative bacillus, not acid-fast. In ooze at 150 fathoms (latitude  $66^{\circ} 32'$  South, longitude  $141^{\circ} 39'$  East), appeared in culture, (a) a Gram-negative, coliform bacillus; (b) a Gram-positive coccus; and (c) a Gram-positive, diphtheroid bacillus. In cultures from 300 fathoms—both made on the "Aurora," north of Queen Mary Land—were found short, thickish, Gram-negative bacilli with a tendency to retain Gram's stain. Finally, a few cultures of mud from 1,520 and 1,700 fathoms, recovered just on the northern fringe of the pack-ice, exhibited no growth.

Apparently, therefore, the oozes contain bacteria in numbers down to a certain variable depth, which has not been ascertained, and certainly bacteria may always be expected, on the continental shelf of Antarctica, down to 300 fathoms, and probably in far greater depths. The fact that the deep-sea trawl used on the "Aurora," during the summer cruise of 1913 to 1914, at eleven stations along the coast of Antarctica, over an east-to-west distance of about 1,100 miles, in depths down to 1,700 fathoms, brought up many forms of marine life on every occasion, and fish on ten occasions, should be mentioned to support the supposition that bacteria were present in the ooze in every instance. Though negative results were obtained in specimens from 1,520 and 1,700 fathoms, a fish, *Chalinura ferrieri*, from 1,700 fathoms, gave a growth in culture, from the intestine, of a Gram-negative, sporing bacillus.

One should quote for comparison the experience of MM. Charles Richet and Portier\*, in their researches in the North Atlantic Ocean; namely, that bacteria were found in deep-sea ooze down to 1,625 fathoms, but beyond that depth they were exceedingly rare or absent.

\* La Troisième Campagne Scientifique de la "Princesse Alice II<sup>me</sup>," par S.A.S. Albert I<sup>er</sup>, Prince de Monaco. Extrait des *Comptes rendus des séances de l'Académie des Sciences*, t. CXXXIV, p. 961. (Séance du 28 avril, 1902).

## CHAPTER IX.

## PHYSIOLOGY.

## I.

THE members of the Australasian Antarctic Expedition were mostly young and in the prime of life. The average age of thirty-seven men (including the land parties at Macquarie Island, Adelie Land, and Queen Mary Land, and the ship's officers) was 26 years. All were of good physique, in sound health, and at some time or another had "roughed it."

In the Hut at Adelie Land eighteen men—two amalgamated parties—lived during the year 1912. As a result of the hard work and wholesome food, the weight of every man increased, on the average, by about 10 lb. In two cases the increase in weight reached 28 lb., and was at its maximum in June and July, the winter months. There was always occupation for everyone outside, assisting in the meteorological, magnetic, tidal, and other observations, while every minute of available calm weather was spent in erecting or repairing the wireless masts. There was no sickness of any kind. Rather one should say that there was a superabundance of health and good spirits, despite the continuous blizzard wind.

It is not surprising, therefore, that the records of blood examinations of six men of the party should show a rise in hæmoglobin values during the period of mid-winter.

Dr. G. P. Howe, of Mikkelsen's Arctic Expedition (1908), notes his experience when wintering on the north coast of Alaska:—

"I kept records of the hæmoglobin of a party of six white men from September to March, covering the whole period while the sun was away. There was no diminution of hæmoglobin attributable to the absence of sunlight."

He goes on to state that similar experiments were tried on the Jackson-Harmsworth Expedition to Franz Josef Land, the Duke of Abruzzi's Expedition, and on Scott's Antarctic Expedition (1901-1904), with the same results.

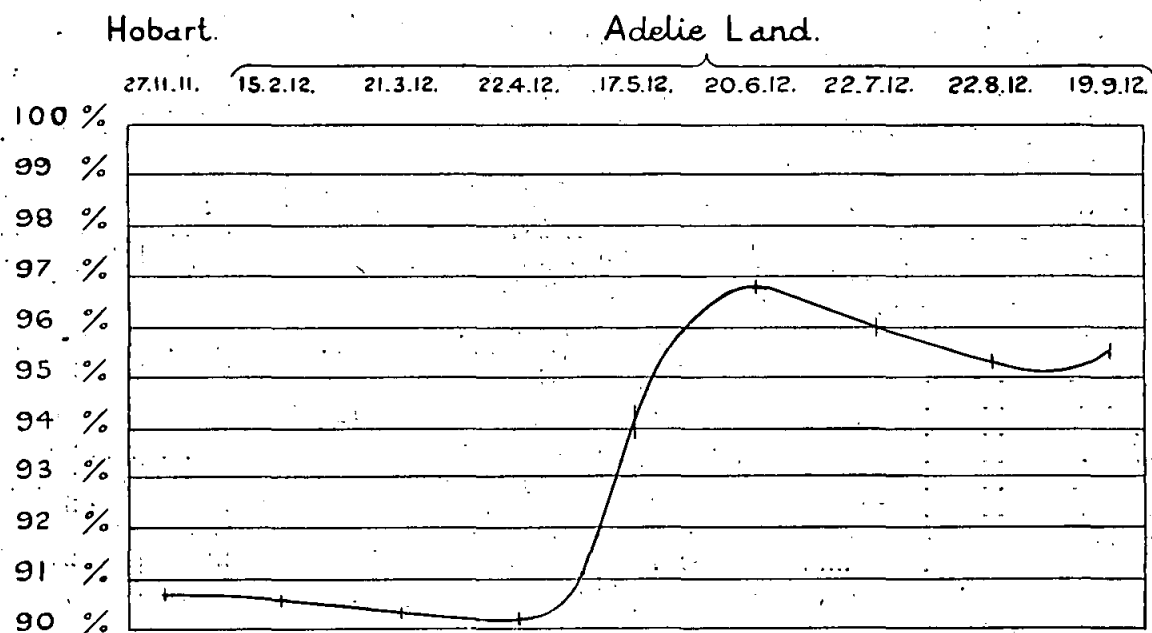
Dr. Koettlitz, during the last-named Expedition, took regular measurements of the weight, the chest, the grip, and the capacity of the lungs of forty-five men of the "Discovery." He observed that the weight tended to increase, though it fell off in a few cases, and that the blood grew richer, with few exceptions.

Six men of the Adelie Land party were subjected to blood examinations and to estimations of blood pressure over a period of ten months, the first tests being made at



Hobart in November, 1911, just prior to the "Aurora's" departure for the South. The details of this work are set out at length below, while two curves, of hæmoglobin values and of blood pressure readings, have been constructed.

## CURVE OF HAEMOGLOBIN VALUES.



The first curve represents the mean of six, monthly values, estimated on nine occasions. It will be apparent that there was, during the first five months, a slight decrease in the hæmoglobin percentage. If reference is made to the numbers of red cells, the hæmoglobin decrease is seen to have been coincident with a rise in the average number of red cells. That is to say, that the sudden increase in the red cells—liable to fluctuation in numbers—may have been responsible for the slight diminution in colouring matter, whose value changes more slowly. The delayed reaction of the hæmoglobin had definitely taken place by the month of May, and had reached its maximum in June, after which it declined slightly for two months, and in September again showed a tendency to rise.

Actually the history of the ten months after leaving Hobart was as follows:—A period of three months of intermittent hard work—the sailor life on the ship, the partial establishment of the Macquarie Island wireless station, the Antarctic cruise, the landing of stores and equipment at Cape Denison, the building of the Hut and the construction of many subsidiary stations for scientific instruments. The red cells increased during the period of muscular activity, totalling in one instance more than 7,000,000. Where the readings were abnormally large, the estimations were repeated.

When the more or less leisured life within the Hut had really commenced, the hæmoglobin value definitely rose in a steep curve. After midwinter the average number of red cells was still on the increase, the hæmoglobin suffering a slight fall, until in August

this average decreased, and the hæmoglobin rose slightly. August and September were months when short sledging journeys were attempted, and the drifting snow was so small in amount that outside work became the rule.

The curve is rather an interesting one when thus related to the physiological life of the six men on whom the experiments were made. Perhaps the most noticeable fact is the high count in red cells which was definitely established after March, 1912.

The number of white cells—almost invariably below the normal—showed, in comparison with the estimations made at Hobart, a slight diminution, maintained until April, 1912, after which the average rose to the original level. The following table shows the mean result of differential leucocyte counts made on six men :—

Name.	Date.	Number of leucocytes.	Neutrophiles.	Lymphocytes.	Mononuclears.	Eosinophiles, &c.
			per cent.	per cent.	per cent.	per cent.
W.H.H. ...	17-5-12	3,437	46.	50	2	2
A.L.M. ...	17-5-12	4,220	41	56	2	1
J.F.H. ...	17-5-12	3,750	51	45	2½	1½
J.G.H. ...	17-5-12	4,062	55	40	2½	2½
E.N.W. ...	17-5-12	5,625	56	38	3¾	2¼
A.J.H. ...	25-4-12	4,687	52	46	2	2
Average ...	.....	4,297	50·1	45·8	2·4	2·8

The absolute number of lymphocytes in this mean count of 4,297 leucocytes amounts to 1,972. In normal blood there are approximately 2,000, so that we may infer that the diminution in the leucocytes as a whole is due to a decrease in the neutrophiles of nearly 3,000 cells. In blood films which were made from time to time, the absence of the usual number of neutrophile leucocytes was noted.

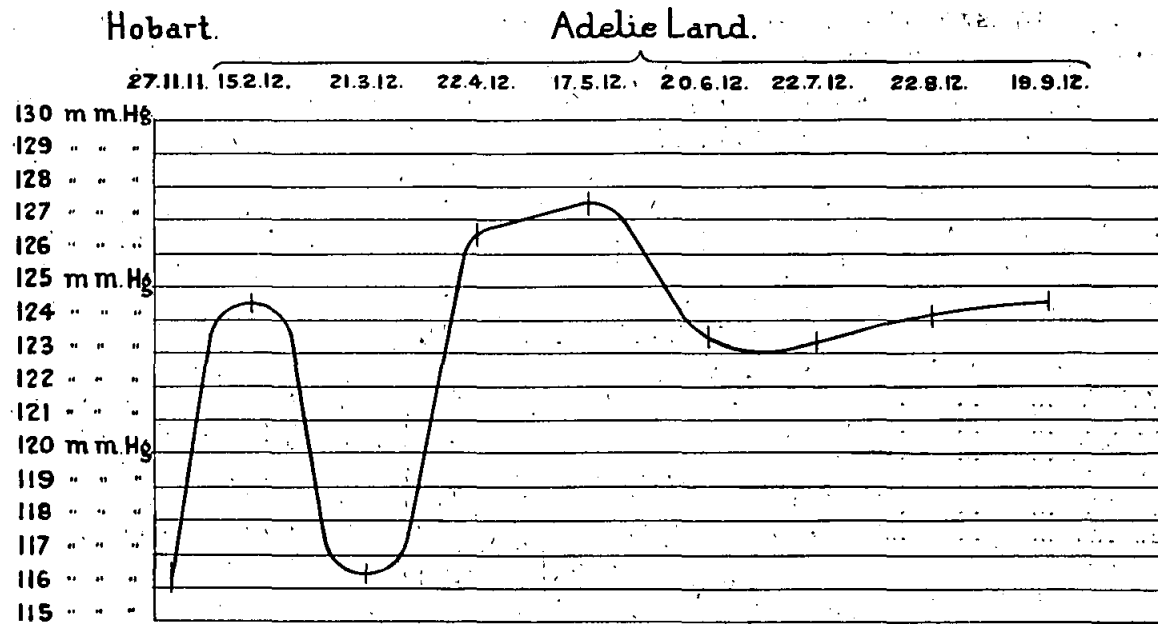
Only on two occasions in the routine blood examinations did the leucocytes show an unusual rise, *e.g.*, A.J.H. on 17th May, 1912, had 10,000 white cells—neutrophiles 81 per cent., lymphocytes 18 per cent.—and complained of a slight “stiffness” of the nose; J.G.H., on 20th June, 1912, had 7,500 white cells, for no assignable cause. On 6th June, 1912, a blood count was done on F.L.S., who was suffering with a whitlow. There were 11,000 white cells, approximately, of which 79 per cent. were neutrophiles, and 20 per cent. were lymphocytes. On 3rd July, 1912, J.C.C. had a dental abscess, with a leucocytosis of 8,125 cells.

I used for the hæmoglobin estimations Haldane's modification of Gower's and Gower's (after Sahli) hæmoglobinometers. Both instruments were compared, on returning to Australia, with the absolute standard—a 1 per cent. solution of ox blood. The picro-carminic jelly of the former hæmoglobinometer showed the same tint as the absolute standard, and that of the latter was very slightly richer, a small correction being applied to the readings whenever this instrument had been used. Another complication which arose was the fact that acetylene light had been used in making most of the observations in Adelie Land, whereas the earlier ones in Adelie Land, and the

preliminary estimation in Hobart had been done by daylight. A large number of comparisons was therefore made of readings in daylight and in acetylene light, the mean difference actually being about 10 per cent. of hæmoglobin value. This correction was then applied wherever it was necessary.

With regard to the curve of blood pressure, an interpretation may be attempted, having due regard to the activities of the six individuals whose mean readings were taken over a period of nine months.

## CURVE OF BLOOD PRESSURE READINGS.



With the unwonted and strenuous work, the blood pressure immediately rose between November, 1911, and February, 1912, suffering a complete relapse in March, 1912. It is difficult to say why this fall should have occurred. Actually, by 21st March, the winter had really started, and our labours were not so strenuous as in the days of hut-building at the latter end of January and the beginning of February. But hibernation was well established in May, by which time the blood pressure had recovered and stood at its highest mean point—more than 127 millimetres of mercury. The next fall was more gradual, and there was a definite upward gradient when work had once more been resumed.

Physiologically one should expect fluctuations in the blood pressure, which depends on immediate factors of environment and physiological function. It is to be regretted that a mercury instrument was not used, as the "spring" gauges are not always reliable. I used a Roger's sphygmomanometer and found it very convenient and portable.

On one occasion I took it with me while on a short, and rather laborious, nine days' sledging trip, with a view to observing if there was any marked difference of blood

pressure at different altitudes. The mean temperature for the period was  $-20^{\circ}$  F., and the average velocity of the wind lay between 55 and 60 miles per hour. The results were as follows, each reading being the mean of three:—

	Sea Level.	1,500 feet.	1,700 feet.	2,000 feet.
F.L.S. ...	121.25 mm. Hg.	125 mm. Hg.	126.25 mm. Hg.	125 mm. Hg.
A.L.M. ...	125 „	122.5 „	137.5 „	142.5 „
Mean of two	123.125 mm. Hg.	123.75 mm. Hg.	131.875 mm. Hg.	133.25 mm. Hg.

That is to say, the mean rise of blood pressure in 2,000 feet is registered as 10 mm. Hg. The readings were taken at the same time of the day—after the evening meal. Such results have little value, as they are isolated observations made under conditions which did not permit of accuracy.

## SUMMARY OF BLOOD EXAMINATIONS.

	Hæmoglobin.	Red Cells.	White Cells.	Colour Index.	Blood Pressure.
No. 1.—Hobart. 27-11-11.					
	per cent.				
A.L.M. ...	88.25	5,680,000	4,687	.77	115 mm. Hg.
J.G.H. ...	92	4,610,000	6,875	.99	114 „
W.H.H. ...	91	5,870,000	5,000	.77	122.5 „
F.H.H. ...	94.25	4,950,000	4,065	.93	119 „
A.J.H. ...	89	4,630,000	5,000	.96	122.5 „
E.N.W. ...	89.5	4,810,000	5,625	.93	103.75 „
No. 2.—Adelie Land. 15-2-12.					
	per cent.				
A.L.M. ...	88.4	5,160,000	3,281	.85	140 mm. Hg.
J.G.H. ...	93	5,310,000	5,000	.87	117.5 „
W.H.H. ...	92.4	6,710,000	3,125	.68	127.5 „
F.H.H. ...	94.25	5,600,000	5,000	.93	136.75 „
A.J.H. ...	85.9	6,930,000	4,062	.61	122.5 „
E.N.W. ...	89.5	5,320,000	5,000	.84	102.75 „
No. 3.—Adelie Land. 21-3-12.					
	per cent.				
A.L.M. ...	86.15	5,525,000	4,375	.77	121.25 mm. Hg.
J.G.H. ...	93.5	4,820,000	3,425	.96	126.25 „
W.H.H. ...	91.4	7,250,000	4,375	.63	115 „
F.H.H. ...	94	5,600,000	5,935	.83	100 „
A.J.H. ...	87	5,000,000	4,062	.87	121.25 „
E.N.W. ...	(?) 90	5,000,000	5,000	.90	115 „
No. 4.—Adelie Land. 22-4-12.					
	per cent.				
A.L.M. ...	88.9	5,780,000	4,220	.78	130.25 mm. Hg.
J.G.H. ...	87.9	5,780,000	5,312	.77	125 „
W.H.H. ...	92.52	6,566,000	3,750	.704	117.5 „
F.H.H. ...	90.4	5,150,000	5,312	.87	127.25 „
A.J.H. ...	91.9	6,350,000	4,687	.72	127.25 „
E.N.W. ...	89.45	6,300,000	5,937	.709	132.5 „

SUMMARY OF BLOOD EXAMINATIONS—*continued*.

			Hæmoglobin.	Red Cells.	White Cells.	Colour Index.	Blood Pressure.
No. 5.—Adelie Land. 17-5-12.							
			per cent.				
A.L.M.	...	...	93.65	5,500,000	4,220	.85	127.5 mm. Hg.
J.G.H.	...	...	93.15	5,920,000	4,062	.78	127.5 "
W.H.H.	...	...	95.15	(?) 7,140,000	3,437	.66	128.75 "
F.H.H.	...	...	95.9	5,830,000	3,750	.82	128.75 "
A.J.H.	...	...	94.4	5,480,000	10,000	.86	130 "
E.N.W.	...	...	92.4	5,210,000	5,625	.88	122.5 "
No. 6.—Adelie Land. 20-6-12.							
A.L.M.	...	...	94.4	5,410,000	4,843	.87	124.5 mm. Hg.
J.G.H.	...	...	93.4	6,460,000	7,500	.72	127.5 "
W.H.H.	...	...	100.15	7,000,000	4,062	.71	122.5 "
F.H.H.	...	...	95.725	6,990,000	4,062	.68	122.5 "
A.J.H.	...	...	100.15	7,060,000	5,000	.709	120 (approx.)
E.N.W.	...	...	96.4	6,000,000	5,000	.803	125 mm. Hg.
No. 7.—Adelie Land. 22-7-12.							
A.L.M.	...	...	94.9	5,880,000	5,625	.807	131.75 mm. Hg.
J.G.H.	...	...	95.9	7,570,000	4,687	.63	132.5 "
W.H.H.	...	...	97.4	6,600,000	5,000	.73	125 "
F.H.H.	...	...	94.9	6,670,000	4,062	.71	115 "
A.J.H.	...	...	98.9	6,725,000	5,312	.73	120 "
E.N.W.	...	...	93.9	6,830,000	6,250	.68	115 "
No. 8.—Adelie Land. 22-8-12.							
A.L.M.	...	...	93.15	5,980,000	5,625	.77	127.5 mm. Hg.
J.G.H.	...	...	95.225	7,500,000	6,250	.63	132.5 "
W.H.H.	...	...	96.9	7,450,000	3,750	.65	125 "
F.H.H.	...	...	93.9	7,300,000	5,000	.64	127.5 "
A.J.H.	...	...	98.4	7,110,000	5,000	.69	117.5 "
E.N.W.	...	...	94.4	6,370,000	4,375	.74	115 "
No. 9.—Adelie Land. 19-9-12.							
A.L.M.	...	...	94.15	6,380,000	4,375	.73	130 mm. Hg.
J.G.H.	...	...	94.65	6,480,000	5,000	.73	121 "
W.H.H.	...	...	97.9	7,860,000	5,937	.62	125 "
F.H.H.	...	...	94.9	6,530,000	5,625	.72	125 "
A.J.H.	...	...	96.9	6,380,000	4,375	.75	130 "
E.N.W.	...	...	94.4	7,050,000	5,625	.66	116.5 "

## II.

## RATES OF GROWTH OF HAIR AND NAILS.

A few observations were made during the winter of 1912, in order approximately to estimate the growth which occurred in nails and hair. The usual plan was to make a small file-mark on the nail, and to measure weekly the distance between this mark and the arc of skin around the matrix. A single hair was isolated by shaving all round it. The measurements were determined with a micrometer, reading to the nearest  $\frac{1}{1000}$  of an inch.

Actually it became very noticeable that the hair of the head and the nails grew so slowly that they were cut much more infrequently than was the case in the temperate climate of Australia. The hair, like other epidermal appendages, tended to become dry

and rather lustreless, because of the low humidity of the atmosphere, the diminished peripheral blood circulation, and the impediment to free exudation of sebaceous secretion. Close-fitting helmets, of course, did not encourage growth of hair over the head. The mean temperature of the Hut was in the vicinity of 40° F., while the mean, annual outside temperature was approximately zero Fahrenheit. The greater part of the winter was spent in the Hut, especially during the long spells of hurricanes, when it might only be necessary to go outside for an hour or more each day to take readings from the meteorological, magnetic and other instruments.

The results are stated as they appear in the Bacteriological Log. The figures (1), (2) and (3) indicate a different nail or hair, as the case may be:—

<i>Nail—left ring finger.</i>			<i>Rate of Growth.</i>
(1) 16-4-12.	Mean of three readings =	.2104 inches	} .0389 in. in 9 days, or .0043 in. per day.
25-4-12.	do do =	.2493 "	
(1) 25-4-12.	Mean of three readings =	.2493 "	} .0426 in. in 14 days, or .003 in. per day.
9-5-12.	do do =	.2919 "	
(1) 9-5-12.	Mean of three readings =	.1919 "	} .0161 in. in 7 days, or .0023 in. per day.
	do do =	.3080 "	
(1) 16-5-12.	Mean of three readings =	.3080 "	} .0359 in. in 7 days, or .0051 in. per day.
23-5-12.	do do =	.3439 "	
(1) 23-5-12.	Mean of three readings =	.3439 "	} .0344 in. in 12 days, or .0028 in. per day.
	do do =	.3783 "	
(2) 4-6-12.	Mean of three readings =	.1897 "	} .0977 in. in 27 days, or .0036 in. per day.
1-7-12.	do do =	.2874 "	
Mean rate of six sets of observations =			.0035 inches per day.

<i>Hair—left frontal region.</i>			<i>Rate of growth.</i>
(1) 16-4-12.	Mean of three readings =	.2335 inches	} .0695 in. in 9 days, or .0077 in. per day.
25-4-12.	do do =	.3030 "	
(1) 25-4-12.	Mean of three readings =	.3030 "	} .0334 in. in 14 days, or .0023 in. per day.
9-5-12.	do do =	.3364 "	
(2) 16-5-12.	Mean of three readings =	.3041 "	} .0644 in. in 7 days, or .0092 in. per day.
23-5-12.	do do =	.3685 "	
(3) 4-6-12.	Mean of three readings =	.2399 "	} .1206 in. in 27 days, or .0044 in. per day.
1-7-12.	do do =	.3605 "	
Mean rate of four sets of observations =			.0059 inches per day.

Hebra (in 1874) estimated the growth of a finger-nail at about 1 mm. (.04 inches) in a week—.0057 inches per day. More recently J. M. H. Macleod has computed the rate at .8 mm. (.032 inches) per day. Granting that the second observation is correct—and without doubt the rate of growth varies according to the latitude, the season, and the mode of life—a nail would appear to grow about ten times as fast in the British Isles as it does in Antarctica.

Schafer states generally that hair grows at the rate of about half an inch in a month, *i.e.*, .016 inches a day. Our own observations, which can only be regarded as very approximate, indicate that hair in a temperate climate grows almost forty times as fast as it does in Antarctica.

## CHAPTER X.

## IMMUNITY.

## I.

BEFORE the departure of the Expedition, the question of testing human immunity by opsonic observations carried out in Australia and in Antarctica opened up interesting possibilities. Thus some weeks were spent in collecting apparatus and in practising the necessary technique. It was obvious, too, that in order to secure evidence of any value, the opsonic index to a definite organism of a person living under civilized conditions would have to be compared with the index to the same organism of the same individual living in the Antarctic regions. To secure such a result, it would be necessary to carry to Antarctica a living culture of a bacterium which would not thrive in the winter quarters selected by the Expedition; or, in other words, a bacterium to which the subject to be examined would not have access in the south. Therefore dried tubercle bacilli and an emulsion of the same organisms were chosen for the purpose of the opsonic tests. *Staphylococcus pyogenes albus* was also used, but the cultures were grown from throat swabbings made in the Hut at Adelie Land, that is, from bacteria to which the individuals examined already possessed immunity.

Under the unusual circumstances of a polar expedition, it had been proved by previous explorers that the ordinary germs of civilization, as those of coryza and influenza, attacked with more virulence than usual the members of an expedition returning home, after their long respite from bacterial invasion. Shackleton relates how the opening of a bale of blankets at Cape Royds, Antarctica, set free organisms which caused a short-lived epidemic of coryza. It was the experience of our own Expedition that men who occupied a certain cabin on the "Aurora," during the first southern cruise, sooner or later contracted influenza, and there were several cases while the ship was within the Antarctic Circle. Perhaps the best example of the liability to bacterial invasion, conferred by living in an apparently germ-free environment, is afforded by the fact that tuberculosis and other diseases have made havoc of Esquimaux tribes in contact with more civilized neighbours. The reason is, because of the close cohabitation of these people, who with a low inherited resistance to tubercle bacilli live together for long winters confined in their igloos of snow.

We had other evidence of this lowered resistance. Whitlows were opened on three occasions during the stay of our party in Adelie Land, and there was one case of dental abscess. The pus from one whitlow grew in culture *Staphylococcus pyogenes aureus*, *Staphylococcus pyogenes albus*, and *Streptococcus pyogenes*. It is to be noted that the first-named organism was not present in the dust of the Hut, as ascertained by monthly plate exposures (Plate 15), nor in pharyngeal and nasal swabbings made on subjects over a period of five months. It is, therefore, tolerably certain that these organisms were latent in the tissues and not local and external in origin. Dr. S. E. Jones

reported that one member of the Western Party had *herpes zoster* during the winter of 1912 in Queen Mary Land. The lowered resistance of nerve ganglia produced by exposure of the individual to intense cold is the only proximate explanation in this particular instance.

Our cultures from various natural sources, and the work of previous observers, have shown that bacteria are widespread in snow, ice, soils, and in the sea, and are present, though reduced in numbers, in the intestines of mammals, birds, and fishes. Of twelve species of bacteria isolated from morainic mud, granite sand, and dried algae, none had pathogenic action when injected into guinea-pigs. Mlle. Tsiklinsky grew from the intestine of a penguin a bacterium allied to *Bacillus pyocyaneus* which had pathogenic properties. Dr. Gazert described a sporing bacillus similar to *Bacillus tetani*, which he obtained from guano and moss soil, but there is no record of its pathogenicity. In Adelie Land seals were frequently seen covered with suppurating wounds, which were proved to contain pyogenic organisms. Still we may not presume that the bacteria of an Antarctic environment, when not of animal origin, are to be regarded in every instance as innocuous.

Small cuts, scratches, abrasions, and frostbites, which were all very common amongst members of the Expedition, did not become infected readily even though neglected. However, it was always found advisable to cover over a wound with a dressing, otherwise it would take weeks to heal, exposed as it often was to very low temperatures. Nails and hair grew slowly, the skin of the hands and face was drier than usual, because of the inhibition or partial solidification of sebaceous secretion, and the peripheral circulation of the blood was decreased in amount. So the edges of a small wound would remain everted and contracted for a long time, and, even though there was a healthy granulating surface, epithelial growth was very tardy.

The results of the opsonic tests in Adelie Land were disappointing, mainly because of the pressure of more important work, and because of technical difficulties which are increased under the rough conditions of an Antarctic hut. Still there were a few notes, and they are stated as they appear in the Bacteriological Log. No comparative estimations were made in Australia.

In six cases where tubercle bacilli were used as the bacterial emulsion the opsonic index was more than unity, and, in one case, it was as high as 2.2. This figure may be explained by the lower phagocytic index in "pool" serum as compared with the same in "own" serum, recorded in each instance. The "pool" or normal serum was a mixture in equal parts of the sera of three individuals. Collecting the blood samples, centrifugalising them and mixing the sera took sufficient time in the cold Hut—with an average temperature of 40° F.—to render them as a medium less favourable for the phagocytic action of the leucocytes. Though the warmest time of the day was usually chosen for the opsonic work, it was impossible, when working with fine columns of fluid in pipettes—and in the absence of an opsonic incubator—to hope for absolute accuracy in the results.



The tests to *Staphylococcus pyogenes albus* (cultured from a throat swabbing) showed nothing of interest, beyond the high phagocytic power, in most cases, of the neutrophile leucocytes to an organism which they were accustomed to combat.

#### OPSONIC RESULTS.

##### *Experimental Observation.*

17-4-12. Opsonic index to *Staphylococcus pyogenes albus*—A.L.M.

The bacterial emulsion was made up from a two days' culture of *Staphylococcus pyogenes albus*. A faint opalescent solution was used.

Phagocytic index = 3.02 in "own" serum.

A "pool" or normal serum was made from three members of the Expedition.

Phagocytic index = .673.

Opsonic index = 4.48.

NOTE.—In all estimations, unless otherwise stated, bacteria were counted in fifty neutrophile leucocytes.

##### *Summary of Estimations.*

20-4-12. The usual procedure was followed. The bacterial emulsion was a faint opalescent solution of a twenty-four hours' culture of *Staphylococcus pyogenes albus*. Blood samples were collected in glass capsules and centrifugalised to obtain serum and washed leucocytes. The "pool" serum was a mixture in equal parts of the sera of three other individuals.

The mean temperature of the Hut was approximately 40° F.

The pipette containing leucocytes, serum, and bacteria, was incubated for fifteen minutes at 37° C.

Opsonic index to *Staphylococcus pyogenes albus*—A.L.M.

Phagocytic index in "pool" serum = .98.

Phagocytic index in "own" serum (vitiated by the presence of small foreign bodies in the mixing pipette).

22-5-12. Opsonic index to *Staphylococcus pyogenes albus*—A.L.M.

Phagocytic index in "pool" serum = 5.0.

(Thirty-six neutrophiles counted.)

Phagocytic index in "own" serum = 17.91.

(Forty neutrophiles counted.)

Opsonic index = 3.58.

NOTE.—A bacterial emulsion slightly less dilute than the one used on 20-4-12, was employed.

24-5-12. Opsonic index to *Staphylococcus pyogenes albus*—J.G.H.

Phagocytic index in "pool" serum = 4.24.

Fifty-three neutrophiles counted.)

Phagocytic index in "own" serum = 9.36.

(Thirty-three neutrophiles counted.)

Opsonic index = 2.25.

Opsonic index to *Staphylococcus pyogenes albus*—J.G.H.

Phagocytic index in "own" serum = 8.9.

Phagocytic index in "pool" serum = 14.48.

Opsonic index = .614.

NOTE.—In this estimation, the mixed fluids (serum, bacteria, and leucocytes) were incubated in a pipette, which contained a small air bubble in the middle of the fluid, accounting probably for the high phagocytic index.

2-5-12. Estimations were made of the opsonic index to tubercle bacillus. The bacterial emulsion was made up from the dried germs. They were ground between two flat discs of glass for four or five hours, filtered, and then diluted with 1.5 per cent. Na Cl to produce a slight opalescent solution.

Opsonic index to tubercle bacillus—A.L.M.

Phagocytic index in "pool" serum = .66.

Phagocytic index in "own" serum = 1.5.

Opsonic index = 2.2.

4-5-12. Opsonic index to tubercle bacillus—A.L.M.

Phagocytic index in "pool" serum = 1.09.

Phagocytic index in "own" serum = 1.7.

Opsonic index = 1.55.

6-5-12. Opsonic index to tubercle bacillus—J.G.H.

Phagocytic index in "own" serum = 1.46.

Phagocytic index in "pool" serum = .84.

Opsonic index = 1.73.

13-5-12. Opsonic index to tubercle bacillus—J.G.H.

Phagocytic index in "own" serum = 1.607.

(Fourteen neutrophiles counted.)

Phagocytic index in "pool" serum = .82.

(Sixty neutrophiles counted.)

Opsonic index = 1.95.

15-5-12. Opsonic index to tubercle bacillus—B.E.S.N.

Phagocytic index in "own" serum = .85.

(Forty-one neutrophiles counted.)

Phagocytic index in "pool" serum = .413.

(Forty-six neutrophiles counted.)

Opsonic index = 2.05.

29-5-12. Opsonic index to tubercle bacillus—B.E.S.N.

Phagocytic index in "own" serum = .888.

(Nine neutrophiles counted.)

Phagocytic index in "pool" serum = .409.

(Twenty-two neutrophiles counted.)

Opsonic index = 2.17.

8-6-12. Opsonic index to tubercle bacillus—A.L.M.

Phagocytic index in "own" serum = .562.

(Sixteen neutrophiles counted.)

(Unfinished.)

Phagocytic tests were carried out in four cases, using a bacterial emulsion of just perceptible opalescence and more dilute than in previous estimations.

28-8-12. Phagocytic index to *Staphylococcus pyogenes albus*—A.L.M.

Phagocytic index in "own" serum = 1.3.

Phagocytic index to *Staphylococcus pyogenes albus*—J.G.H.

Phagocytic index in "own" serum = 1.13.

(Twenty-three neutrophiles counted.)

28-8-12. Phagocytic index to *Staphylococcus pyogenes albus*—A.L.M.

Phagocytic index in "own" serum = 1.18.

30-8-12. Phagocytic index to *Staphylococcus pyogenes albus*—J.G.H.

Phagocytic index in "own" serum = .82.

NOTE.—This estimation was vitiated by the incubator being at 38° C. for the fifteen minutes during which the pipette was inside it.

## II.

With a view to observing whether there was any diminution or disappearance of bacteria grown under ordinary conditions in cultures from the mouth, throat, nose and skin of members of the Expedition, examinations of swabbings were made over a period of nine months; from the time of leaving Tasmania (December, 1911), until the latter end of the first Antarctic winter (August, 1912). The following are the results:—

Hobart. 27-11-11.

A.L.M.	Throat	...	{	Staphylococcus pyogenes aureus.
				Streptococci.
	Mouth	...	{	Staphylococci (white in culture).
				Streptococci.
	Nose	...	{	Staphylococcus pyogenes aureus.
				Staphylococci (white in culture).
				Diphtheroid bacilli (K.L.B.).
W.H.H.	Throat	...	...	Staphylococci (white in culture).
	Mouth	...	...	Staphylococci (white in culture).
	Nose	...	...	Staphylococci (white in culture).

Hobart. 27-11-11—*continued*.

J.G.H.	Throat	...	{ Streptococci.
		...	{ Bacillus hoffmanni.
	Mouth	...	{ Staphylococci (white in culture).
		...	{ Staphylococci (white in culture).
	Nose	...	{ Bacillus hoffmanni.
		...	{ Streptococci.
J.F.H.	Throat	...	{ Staphylococci (white in culture).
		...	{ Staphylococci (white in culture).
	Mouth	...	{ No growth.
		...	{ Staphylococci (white in culture).
	Nose	...	{ Staphylococci (white in culture).
		...	{ Streptococci.

## Adelie Land. 20-2-12.—

A.L.M.	Throat	...	{ Streptococci.
		...	{ Staphylococci (white in culture).
		...	{ Diptheroid bacilli (K.L.B.)
	Mouth	...	{ Staphylococci (white).
		...	{ Staphylococcus pyogenes aureus.
	Nose	...	{ Staphylococci (white in culture).
		...	{ Bacillus hoffmanni.
		...	{ Staphylococci (white in culture).
	Throat	...	{ Streptococci.
W.H.H.		...	{ Bacillus hoffmanni.
	Mouth	...	{ Bacillus subtilis.
	Nose	...	{ Staphylococcus pyogenes aureus.
		...	{ Staphylococci (white in culture).
	Throat	...	{ Staphylococci (white in culture).
		...	{ Streptococci.
J.G.H.	Mouth	...	{ Staphylococci (white in culture).
		...	{ Staphylococcus pyogenes aureus.
	Nose	...	{ Staphylococci (white in culture).
J.F.H.	Throat	...	{ Staphylococci (white in culture).
		...	{ Streptococci.
	Nose	...	{ Staphylococci (white in culture).
	Mouth	...	{ Staphylococci (white in culture).

## Adelie Land. 25-3-12.—

A.L.M.	Throat	...	{ Staphylococci (white in culture).
		...	{ Streptococci.
	Axilla	...	{ Staphylococci (white in culture).
	Nose	...	{ Bacillus hoffmanni.
		...	{ Staphylococci (white in culture).
W.H.H.	Throat	...	{ Streptococci.
		...	{ Staphylococci (white in culture).
	Ear	...	{ Staphylococci (white in culture).
	Nose	...	{ Staphylococci (white in culture).
	Throat	...	{ Staphylococci (white in culture).
	Axilla	...	{ Staphylococci (white in culture).
J.G.H.	Nose	...	{ Staphylococci (white in culture).

Adelie Land. 25-3-12—*continued*.

J.F.H.	...	Throat ...	...	{ Staphylococci (white in culture). Streptococci.
		Nose ...	...	Staphylococci (white in culture).
		Ear ...	...	No growth.

## Adelie Land. 26-4-12.

A.L.M.	...	Throat ...	...	{ Staphylococci (white in culture). Streptococci.
		Nose ...	...	Staphylococci (white in culture).
J.G.H.	...	Throat ...	...	{ Staphylococci (white in culture). Streptococci. Bacillus hoffmanni.
		Nose ...	...	{ Staphylococci (white in culture). Bacillus hoffmanni.
J.F.H.	...	Throat ...	...	Staphylococci (white in culture).

## Adelie Land. 26-5-12.

A.L.M.	...	Throat ...	...	{ Staphylococci (white in culture). Streptococci.
		Nose ...	...	Staphylococci (white in culture).
J.G.H.	...	Throat ...	...	{ Staphylococci (white in culture). Bacillus hoffmanni.
		Nose ...	...	Staphylococci (white in culture).
J.F.H.	...	Throat ...	...	{ Staphylococci (white in culture). Bacillus hoffmanni.
		Nose ...	...	Staphylococci (white in culture).

## Adelie Land. 30-6-12.

A.L.M.	...	Throat ...	...	Staphylococci (white in culture).
		Nose ...	...	{ Streptococci. Bacillus hoffmanni.
J.G.H.	...	Throat ...	...	{ Staphylococci (white in culture). Bacillus hoffmanni.
		Nose ...	...	Staphylococci (white in culture).
		Skin (face)	...	Staphylococci (white in culture).

## Adelie Land. 31-7-12.

A.L.M.	...	Throat ...	...	Staphylococci (white in culture).
		Nose ...	...	Staphylococci (white in culture).
J.G.H.	...	Throat ...	...	{ Staphylococci (white in culture). Streptococci.
		Nose (?)	...	Staphylococci (white in culture).

## Adelie Land. 31-8-12.

A.L.M.	...	Throat ...	...	Staphylococci (white in culture).
		Nose ...	...	{ Staphylococci (white in culture). Bacillus hoffmanni.
J.G.H.	...	Throat ...	...	Staphylococci (white in culture).
		Nose ...	...	{ Staphylococci (white in culture). Bacillus hoffmanni.

The following table shows the incidence of the different species of bacteria in swabbings (throat and nose) from A.L.M. and J.G.H. over the period of nine months :—

Organism:		Nov. 1911.	Feb. 1912.	March.	April.	May.	June.	July.	Aug.
A.L.M.	Staph. aureus...	2	1	...	...	...	...	...	...
	Staph. (white in culture) ...	1	2	1	2	2	1	2	2
	Streptococci ...	1	1	1	1	1	1	...	...
	Bacilli ...	1	2	1	...	...	1	...	1
J.G.H.	Staph. aureus...	...	1	...	...	...	...	...	...
	Staph. (white in culture) ...	1	2	2	2	2	2	2	2
	Streptococci ...	2	1	...	1	...	...	1	...
	Bacilli ...	2	...	...	2	1	1	...	1

It may be noted from the table that there was a certain tendency of the bacteria to fall off, though no generalisation could be made from such meagre results. It was impossible through lack of culture media to take swabbings from more subjects, but it became evident at least, after March, 1912, that *Staphylococcus pyogenes aureus* did not appear on any cultures. Additional swabbings from other members were occasionally taken to secure the organism, as it was intended to use it for opsonic observations. The dust of the Hut, with its eighteen inhabitants, swarmed with *Staphylococcus pyogenes albus*, other white staphylococci, bacilli of various kinds, and many varieties of moulds; but on agar plates exposed monthly over a period of six months (March to September, 1912), *Staphylococcus pyogenes aureus* never appeared among the colonies. Apparently it is not so resistant to cold, being thermophilic in habit.

### III.

13-4-12. A culture was made from a small boil on the arm of F.L.S., which, after forty-eight hours at 37° C., showed white, circular colonies fusing in a continuous growth. *Staphylococcus pyogenes albus*.

14-4-12. An agar plate, which was exposed for twelve hours near the roof of the Hut at night, grew moulds of various colours (*Penicillium*, *Eurotium*, *Mucor*, &c.), many colonies of *Staphylococcus pyogenes albus*, *Bacillus subtilis*, and other bacilli. Throughout the winter, for a period of six months, monthly exposures of agar plates were made in the Hut at night, and, according to counts made of the colonies, the number of organisms was markedly on the increase. (Plate 15.)

No aerial exposures of culture media were made outside the Hut or on the glacier, since the air was seldom still for more than a few hours at a time in the winter, and the whole of the short summer was spent in sledging.

6-6-12. F.L.S. developed a deep-seated whitlow, which was opened under an anaesthetic. Cultures were made from the pus and *Staphylococcus pyogenes albus*, *Staphylococcus pyogenes aureus* and *Streptococcus pyogenes* were grown.

3-7-12. Cultures were made from a tooth abscess and the following bacteria were isolated :—*Staphylococcus pyogenes albus*, *Streptococcus pyogenes*, diphtheroid organisms and *Bacillus hoffmanni*.

## CHAPTER XI.

## DIETETICS.

THE subject of dietetics is one that must be taken seriously on a polar expedition. It is necessary to have food in variety as well as in great quantity. At the present time, provisions which have been preserved by tinning or drying may be carried to the South and kept indefinitely in a frozen condition. The preliminary essential of which to be certain is that there is no bacterial or other contamination. In these days of scientific method, the preservation of food has become a fine art, and scurvy should never be due to technical faults in its preparation.

Scurvy is a disease which occurred in the past as a result of a long-continued régime on the same ration, or because of chemical deterioration in the ration, or on account of a combination of the two causes. However, the most modern view inclines to the belief that if there is a deprivation of certain constituents of diet—notably the so-called vitamines—scorbutic symptoms will tend to arise if these vitamines are denied for a certain period, especially if the body is at the same time being subjected to the intense fatigue of laborious work. On the extensive sledging journeys which have been prosecuted by polar expeditions, these conditions are often present, and even recently scurvy has been reported. It is too much to expect of human endurance that a man should live for three months or longer on the more or less artificial sledging ration, doing severe and continuous muscular work throughout extremes of low temperature, without incurring some constitutional risk.

Quoting our own experience with the Australasian Antarctic Expedition, there were certain physiological lessons which we learnt during sledging journeys. One of the most important was the inadequacy—under certain circumstances—of the 34 ounces which constituted the daily ration. Inadequate they were to satisfy hunger completely, or to repair the tissue waste, if the work were abnormally severe. If all were going well, the temperatures were high, and the work was moderate, sledging life seemed rather pleasant, and one had, at the end of the day, that comfortable sense of repletion which comes to the labourer in a civilised country. Individuals and appetites vary a good deal, but he was a difficult person to satisfy who did not settle down to the routine, and who did not, from daily habit, regard the amount and quality of the food as all-sufficient for his needs. And in this regular discipline we have the key to many of the great feats of endurance which have been accomplished by explorers. But in this same discipline, we have the explanation of the hidden danger which may not be apparent in the sunny weeks of the outward journey, but which may become a real menace when superhuman effort is required, and the point is long past when repair and waste are fully met by the food absorbed.

The evidence of discipline in a reinforcement of the will is sometimes not apparent, until one has regained winter quarters and has been confronted with provisions in plenty and in variety. Then the pent-up appetite, satisfied hitherto on dietetic principles, is, so to speak, released to indulge spaciouly. That is to say, the usage of a lifetime will assert itself, despite those relative percentages of protein, fat and carbohydrate fully supplied by the sledging ration.

The discipline of sledging is made tolerable by many factors of environment as well as by the exhibition of character. There are the novelties of every day, the glimpses of new land, the compassing of latitude and longitude, the full-blooded healthfulness, the resilient optimism, the unwavering comradeship, the individual idealism, which help to make light the grinding routine, so that the satisfaction of an unwonted and abnormal hunger is always subservient to the cause of scientific exploration. Still we may never forget that it is an animal we are feeding, and that though he be inured to privation, he will, inevitably, follow his natural bent.

In Adelie Land, during the springtime sledging, the three parties who went out on the plateau, while the temperatures were low and the wind was very high, consumed the full ration and many "extras," without any physical inconvenience. During the summer, the Eastern Coastal Party, encountering fairly high temperatures on sea ice, experienced slight indigestion on account of the fatty "hoosh." They perspired a good deal and, in consequence, drank as much liquid as possible to assuage their thirst. Later, when colder conditions set in and the work was still arduous, the ration did not rise to the fullest requirements. On returning to the Hut, fruit and fresh penguin eggs seemed to them the most desirable articles of food. The Southern Party worked back towards the south magnetic pole, at a height of 6,000 feet. At first, in the superabundance of "good condition," they were unable to eat the full ration, but, after a few weeks of heavy work, they found, in increasing measure, that it did not meet their demands. They returned with avidity, at the Hut, to a diet of fresh penguin meat, penguin eggs and fruit. Dr. Mawson, during his eventful and tragic journey, lived for nearly two months on dogs' meat and a very scanty ration. He suffered from starvation primarily, though he relates that he had cutaneous eruptions accompanied by marked desquamation of the skin and loss of hair. For some time, after arriving at the Hut, his digestion was impaired and he had a partiality for farinaceous foods, fruit and eggs.

Of course, in polar sledging, the explorer is faced with the difficulty of being unable to replenish his food, and by a system of depot-laying is enabled to cover long distances. The provisions he uses must be of the best quality, and, for economic reasons, in a dry condition. The problem of travelling in Antarctica was first satisfactorily solved by Captain Scott, who drew on the experience of previous Arctic explorers, notably Nansen. Captain Scott has been the pioneer of the South in the elaboration of details of equipment, and in the finest possible adjustment of diet to



energy expended. It is experience, always, that teaches. The evolution of the polar sledging ration has been marked by the scientific effort to supply in their due proportion those articles which are necessary for the bodily maintenance of the hard working sledger. The adaptation has been, in part, artificial, since no manufactured food can take the place of fresh, cooked meat; vegetables and fruit of the garden, the accustomed loaf of bread, and the eggs and milk of the farm. It is when the man is worn down to a threadbare resistance, and yet will adventure in the face of great odds, that the inadequacy of the sledging ration is most obvious and the danger of scurvy is most imminent. There are limits to the capacity of the human machine.

Actually the sledging ration has been narrowed down to almost two pounds a day. McLintock in 1850 allowed 42 ounces, Nares in 1875, 40 ounces, and the Duke of Abruzzi in 1900, 43·5 ounces per day. Since the days of these explorers, water has been eliminated more and more from the ration, and the relative amounts of protein, fat and carbohydrate have been readjusted. Scott in 1903 used a minimum of 34·7 ounces, Shackleton in 1908, 34·82 ounces, and our own Expedition in 1911, 34·25 ounces a day.

To come down to details, in Adelie Land the sledging ration apportioned to one man for a day was composed as follows:—Plasmon biscuit, 12 ounces; pemmican, 8 ounces; butter, 2 ounces; plasmon chocolate, 2 ounces; dried milk, 5 ounces; sugar, 4 ounces; cocoa, 1 ounce; tea, 25 ounces. On the assumption that one gramme of protein or carbohydrate yields 4·1 calories, and the same amount of fat produces 9·3 calories, the fuel value of this dry ration has been computed at approximately 5,136 calories (plasmon biscuit, 1,428 calories; pemmican, 1,516 calories; butter, 418 calories; plasmon chocolate, 326 calories; dried milk (Glaxo), 804 calories; sugar, 464 calories; cocoa, 176 calories; and tea, 4 calories). This is a high figure, ranking with the potential energy of the food consumed by a blacksmith or a navvy. Hutchison supplies a scale which is interesting for purposes of comparison:—

Rest ( <i>e.g.</i> , clerk at a desk)...	2,500	calories.
Professional work ( <i>e.g.</i> , a doctor)...	2,631	„
Moderate muscular work ( <i>e.g.</i> , a house-painter)...	3,121	„
Severe muscular work ( <i>e.g.</i> , a shoemaker)...	3,659	„
Hard labour ( <i>e.g.</i> , a blacksmith or navvy)...	5,213	„

A large proportion of fat is supplied in the pemmican (mixed with 50 per cent. of pure lard), the butter, the plasmon chocolate and the dried milk. Fat is a necessity in an Antarctic dietary. The prodigious amount of blubber consumed by Esquimaux races is evidence at once of its particular value in a cold climate. Our own experience showed how appetite was stimulated by dishes containing a large quantity of fat. "Hoosh"—a hot porridge-like mixture of pemmican, lard, and biscuit—was the substantial food, night and morning, "on the trail," and the solid butter was a delicacy which was eaten by every sledger, for its own sake.

The importance of heat, as well as a heat-producing food, is appreciated after a day's work. The tent is pitched, the footwear (finnesko) are changed, and the meal is cooked and served hot. After the exertion of hauling the sledge for many hours, the circulation of the blood decreases in force, and is often insufficient to warm the feet and hands, especially if the temperature of the air is unusually low. It is then that the first few sips of hot liquid stimulate the heart, and produce after a short time an intense glow all over and through the body. When the meal is finished, the sledger "turns in" to his fur sleeping-bag, thereby conserving precious heat which might be otherwise quickly lost by radiation. If the weather is warm, and the sledger is well-fed and in good condition, he will sleep, warm, throughout the night. If his superficial fat has been lost by overwork, and the atmospheric temperature is sufficiently low, he will wake up, after some hours, with cold feet. The amount of ingested food, the degree of muscular work, and the bodily reaction to environment are often nicely balanced in their relation to one another.

Of the carbohydrates, sugar in particular is a fuel easily and quickly assimilated by the working muscles. Chocolate was universally popular amongst the parties who explored Adelie Land and King George V Land during the summer, 1912 to 1913. Cheese, of higher calorific value, might have taken its place, if the general preference had not been for sugar. Morning and evening meals consisted of "hoosh" and cocoa, while the midday lunch was made off biscuit, chocolate, butter and tea (containing dried milk). The morning's work might have been strenuous, but the sweetened tea and the chocolate reduced perceptibly the limb-weariness. It was the custom of some of the men to keep a small reserve of chocolate for the late afternoon, to nibble in the spells between fatiguing periods of continuous pulling. The consequent relief was always noticeable.

The protein *pièce de résistance* of the sledging dietary was the pemmican or powdered dried beef; next, in order of importance, being the gluten and plasmon (casein) content of the biscuits, and the casein of the dried milk and chocolate. Protein has been described as "quick fuel," and there is no doubt that if personal inclination is of any physiological account, the sledger feels that his breakfast and dinner are his chief sustenance and mainstay. At the Hut, during the long winter, penguin and seal meat were eaten in rotation three, and often four, times a week. Frozen mutton was a "treat" for Sunday, and on the other days stews and curries of tinned meat were the rule. Tinned fish was ordinarily consumed at lunch, while the breakfast of fruit, porridge, bread or scones, was a "light beginning." There is no doubt that penguin and seal meat were relished more and more as the skill of the amateur cooks increased. In fact, the penguin steak fully took the place of the beefsteak of civilization. The Eastern Coastal Party, at one stage in their journey, encountered a rookery of penguins, which made a very welcome innovation for some weeks in the monotonous, but always appreciated, ration. Seal blubber, even in Antarctica, is always nauseous at first, and it is only as a matter of necessity that one will, with the "sauce" of a wolfish appetite,

acquire a liking for it. The first distaste for seal meat is mainly due to the small lobules of fat, which should, before cooking, be separated from the muscle as far as possible.

Hutchison quotes a standard of the different nutritive constituents which are required each day by a man, of average build and weight, doing a moderate amount of muscular work. Of protein there are 120 grammes, fat 50 grammes, and carbohydrate 500 grammes; a total of 670 grammes—in terms of energy, 3,007 calories.

For purposes of comparison, we may set out the relative amounts of protein, fat and carbohydrate in the daily sledging ration; 310 grammes, 237 grammes and 403 grammes, having as heat value 5,136 calories, approximately. In this calculation, 11 grammes of water, contained in 56.6 grammes (2 ounces) of butter, are neglected, the rest of the ration being regarded as dry. It is obvious that, when due regard is paid to the much larger amount of food consumed (950 grammes), the relative quantities of protein, fat and carbohydrate, according to the normal proportions stated above, should be 170, 70, and 710 grammes. That is to say, in Antarctica, the tendency is to reduce the carbohydrate, and to increase both fat and protein; the former in greater measure than the latter.

The question has been debated as to the use of alcohol on polar expeditions. Whisky, port wine and claret were used very sparingly by the party at Commonwealth Bay; in fact it was only on occasions for celebration that alcohol was drunk, and then the small stock of luxuries, including jugged hare, asparagus, sweetmeats, cakes and fancy biscuits, were also drawn upon. There is no doubt that these special dinners, which occurred monthly, had, on the whole, a very beneficial effect. The psychic factor is to be reckoned with during a long polar winter, so that anything in moderation, which assists to "raise the tide of life," such as a jovial, social gathering—and its accompaniments—must always be a great good. The Southern Sledging Party, during their return journey from the vicinity of the south magnetic pole, failed to find a depôt 67 miles from the winter quarters. They (three men) were faced with the necessity of travelling this distance, their remaining food being a few negligible scraps of biscuit and chocolate. Fortunately, they had, as well, a small store of absolute alcohol, which was ordinarily used for lighting the primus stove. Without doubt, the stimulation of small quantities of the alcohol, taken from time to time, helped them in safety to the Hut.

## CHAPTER XII.

## PSYCHOLOGY.

A POLAR expedition is always invested with a certain glamour in the minds of those who merely witness its departure, enthuse over its exploits, and welcome it back to civilization. The ends of the earth are plunged in a mystery which is regarded by ordinary folk with an indefinable and irrational wonder. Even the mariner of the cold southern seas, who careers through the perils of wind and ice in the steel sailing ship of to-day, fails to pass by a mental process of simple extension to the frozen plain of solid water, and to the scanty beacons of dark land in the wilderness of white. His simplicity of outlook, his rigid acceptance of stark actuality, and his deepening mysticism are the impediments to a sane understanding. Even the experienced traveller who has rounded the Horn and viewed Iceland and Spitzbergen, breathing more than the atmosphere of the ice-world, will do homage to one who has seen the implacable polar night and the phenomenal glories of the weird Aurora. Curiously enough, it is often the man of the world, in a local sphere, who has read widely and passed shrewdly in review a multitude of characters, who will appraise the explorer at his true worth. Or again the thinker—a scientist and a recluse, maybe—who has lived through the imagery of books in the very fastnesses of the waste places, will be most competent to judge the rationale of polar-expeditions.

The history of Arctic exploration goes back for many centuries to the ancient Thule of Pytheas of Massalia. The Antarctic regions, slumbering for generations as the Terra Australia of Finné, have only been approached since the days of Cook. Few, comparatively, have been the men who have gone north and south, great have been the privations and profound has been the tragedy of the lost, glorious has been the achievement, and far-reaching have been the discoveries. So the world of humanity, wrapped in its own concerns, has merely witnessed a succession of sidelights, illuminating aspects of the whole. Therefore they will welcome the explorer as a kind of curio, a being who will unravel unseen things, who will hold them like the Ancient Mariner in the tense grip of adventurous narrative, and transport them irresistibly to the sphere of the unseen. Ignorance—we may say blank ignorance—is the foundation of this popular wonder.

It is scarcely just to expect the busy man of the city, the naive rustic of the country, to be otherwise than natural in the face of the unknown. He is curious, he is anxious to widen his knowledge, he has, unless dormant and circumscribed, an innate love of the vaster limits of his globe; one with the idealism which tints his conception of stars in an infinite universe. And it is this ideal element, greater or less in measure, which determines the unconventional life of an explorer, and which often leads his admirer to blind hero-worship.

There are in the mind of every discoverer sailing for unseen lands, various motives impelling his action. There is the scientific passion for the irradiation of truth—a longing to go to the root of the matter and to know ultimate causes. The love of a life of action, where will and muscle are strained to the utmost, where the conventions and guises of artificialised surroundings are thrown aside for the crude, chill embrace of stark realities, was immanent in Columbus just as in Captain Scott of these modern days. The fervour to share an adventure with another heroic soul, to be locked in a grand enterprise with men of like aspiration; the emulation, the good fellowship, the sharing of self in true lineaments of character, the corporate sense of a worthy achievement carried through vicissitudes to that small pinnacle of human success; all these make the romance of exploration. There are some who burn the still flame of pure science, and who think in terms of a geographical quest, a geological triumph, a biological paradise; but they usually end in finding their own selves. There are youths afire with the devilry of daring who would win their spurs in a headlong rush, and these have steadied to veterans, young and controlled, with respect for ability and a new vision of the infinite bounds of knowledge. But all, novices and men of experience, sailors, soldiers and students, specialists in trade and profession, wanderers and learned recluses—all in their panoply—have sipped or drunk deep at the chalice of adventure.

#### KNOWLEDGE.

The premonition of great things to be disclosed is so strong and insistent that the whole of consciousness is keyed up to live in a new "universe of discourse." To know, to feel and to will are to be tintured with novel colours of association, and the ethical man is to encounter diverse personality among his comrades, and Nature in many moods, lovely, austere and neutral. He will be called to respond to his environment, he will be rebuffed or treated with indifference; he will end by relating his consequent morality to the code of everyday—the human code obtaining among his fellow men.

We may never analyse the elements of primal sensation as they are registered in the consciousness of a child who opens his faculties to the early vibrations of the world. The foundation of working, responsive mind is laid long before the intellect soars as a free agent, turning upon itself a prying gaze of recognition, marking, classifying, correlating and building up its science of psychology.

Still, there is in the first sudden impression of the ice-world, striking with novel force the adult consciousness of the explorer, something akin to what we may imagine is contained in that initial experience of the child. He is, maybe, a dweller in cities, small in geographical conception, unaccustomed to the nostalgia of a wide sweep of landscape or a sequestered glade in the forest. The sensation is, therefore, the more abrupt and intense, the "spread" over and through consciousness the more enveloping and intimate, and consequently the memory of its accession the more retentive and accurate.

The first iceberg, looming up ghostly and pallid over a grey, heaving sea in the stillness of some chill, foggy zone where misty vapours hang motionless above the water, makes a deep imprint on an expectant mind. Or the flash of an alabaster mountain dripping in the green seas, pierced by shafts of sunlight—azure caverns torn in its snowy flanks, the turmoil of surf across the emerald ice-foot, the pinnacles and battlements in a sheen of light—all this is ineradicable!

Sights and sounds crowd in through the senses as wonder follows wonder, until sense-perception tires and passes to its apperceptive phase.

The world of the pack-ice has succeeded the bergs afloat in the blue solitude of the sea. The still ocean is littered with white fragments, giant monoliths, huge bastions, broken columns, flattened acres and dishevelled rubble of ice, stretching to a dim southern horizon, the azure of the sky's cope pallid in a motionless blink.

The eye is swift to connote. The ship's massive bow grinds into the floes and they glide past in growing detail—their sunken bulk shadowy green beneath the water line, the billowy beds and clods of new-fallen snow, the compact sheen of the polished surface, and the green splinters of sea-ice striking upwards in the pressure ridge! Perception lingers on the unique—the veins of golden yellow lining the upturned bergs—the frozen life of the diatoms—the chinks of dark, vaporous blue chiselled in the marble façades, the horizontal waving lines of annual snow, the red shrimps clustering in the crystal water, the long-drawn blow and foaming swish of the whales, the torpid ease of the bulky seals, and the statuesque half-human pose of the penguins. The gelid air, the pure sun, the radiant whiteness and the mobile water, amid the immensity of solitude, flow in gradually to the consciousness, amid the streams of novelties, as an omniscient impression.

Associations of perception and idea have been quickened. Antarctic life seems quaint and bizarre in the biological scheme. The white expanse is an infinite wilderness, cold, pitiless and incomprehensible. The scenes of polar expeditions, visualised in picture and graphic narrative, receive recognition, and challenge individual interpretation. The icy walls sculptured, haphazard it seems, in the perfection of nature, recall the design of some human monument of civilization; the glancing shaft of an ivory pinnacle conjures up an Oriental minaret; the sublimity of this maze of architecture, far from the haunts of men, seems a prodigal waste, in the stricture of a commercial mind.

The days and weeks pass by, bringing their wealth of undreamt experiences, until one has reacted to environment, accepting the kaleidoscope of shifting images as inevitable. Reason and imagination have been silently at work reconstructing the basis of ideas, extricating the false concept, discarding the preconceived notion, rejecting false associations, propelling by an intellectual force the winnowing current of scientific knowledge and criticism. But still the synthesis of new-found facts is being made, and ever and anon the mind is subjected to the shock of some salient feature, some fresh objective.

The evening of a day approaches when the sun of midnight rides low over the snowy ridge of the upspringing plateau. The torn and writhing glacier suffused with palest lilac, surges down and stands in the sheerness of night as a steel-blue wall, fronting the sea that glitters in the slanting sun. The sun has dipped into the cold contour, and the warm colour and sparkle die away. The plateau has grown more immense, more motionless and mystical. Imagination reaches back to the earthly confine of that vast upland plain of crystal thickness overriding the polar height, and sheathing the frozen cap of the hemisphere. And imagination, as it will when confronted by an overawing spaciousness, soars to a supernatural explanation, and there is born the essence of religious thought; one with the sense of mortality that springs from contemplation of a star beyond the human orbit. The existence of an incomparable Divinity becomes a rational and unimpeachable challenge.

#### FEELING.

Accompanying our comprehension of that sudden and picturesque debut into an inconceivable world of charm and changing wonder, there are the several thrills of superadded sensations, the tingling recognition of apperception and the warm motions of fresh ideas. But perhaps mere æstheticism grows chill, beside the ardour and delight with which one may share with another comrade these first rapt glimpses into virgin solitudes.

One stands beside us on the deck. He has lived the rough, wild life with its surfeit of freedom. He has trodden the snows of a thousand miles over that uprising ridge, standing far back behind a waste of ice-fettered water. He has told us of those evanescent sky-lines mounting in endless undulations to the acme of desolation; he has related the inspiration of those dazzling heights, the endless trudge, the jarring strain, the sweating discipline of that onward march; we have shuddered at the calm tale of unblenching heroism, we have shared instinctively in the uplift of some invincible dream of a man's conquest; we have lain down by the evening fire of contentment, and throbbed in the warmth of human effort realised; we have felt the hint of an intangible thing—the dumb shaping to a glorious, immanent Divinity. In the glowing sense of camaraderie there is the solidest, the most tangible grip on consciousness in feeling.

Another scene. A picture of heavenly hues has awakened anew the sensations. The sun of stormy winter sets in the vibrant gold washed along the northern horizon. The wind has souged to the calm as of an immense cathedral of snow and sea, within a canopy of sky. Bergs in shapely mirage swim in the liquid rose of the far-away ocean, glancing up the palest lilac to the dome above. The zenith is suffused as impenetrable violet, arching down to meet the icy pallor of the inscrutable plateau. The water is rigid in spreading, frozen plaques—a mirror of the changing shades of sunset colour. It is a scene which spells in feeling—the breathless, inward surge at the marvel of unassuming loveliness, and the exquisite workmanship of matchless creation.

## WILL.

Perhaps there is no element in consciousness—though we may not rationally single one out—which receives stronger curb or more decisive strengthening than that of will. From the first, the explorer contends with difficulty; he is thrown on his own originating resources. He is deceived by the ease of his own daring mind, he finds he has failed from neglecting sane foresight, but nevertheless he must win through to the semblance of success.

In the battling days, when a home is won within the snowy maze of a land blotted out by racking blizzards, each one is testing his juvenile strength. There is the buoyancy of the beginning, the glamour of the far-away end. We may laugh at small adversities, knowing that to endure many is to rise to the culmination of mental force which will succeed against the formidable power.

The sledger makes off with his boon companions to seek out the waste places, to test his new-found strength against the stern, foodless, cold-gripping wilderness. The sun shines on the prospering days, the sledge glides merrily, a hummock of black land looms up, and the geography of an unknown latitude has been widened. But the trail leads unerringly to danger. And danger, once viewed frontwise with open eyes, vanishes to the contingency of every day. The experience of small perils is the education to a bland contemplation of horror in its just actuality. The habit of many days has ceased to be mere adventure. It is when that habit is rudely broken by the fell shock of calamity, that the slumbering sea of volition boils up to prove its strength.

There is the vision of a figure stumbling, companionless, dragging on through the changeless days of threshing, seething snow-drift. He has learnt in times long past the lessons of adversity, the grand solitude of self-reliance. He is impelled to stumble on, sinking in the yielding beds of downy snow—so white and pure, yet so relentless in its mockery of human suffering! Hands and feet numb to the flapping gusts of the sleeting blizzard; yet the heart palpitates hot in the will-driven frame of the man who fights for the life still sweet to self, who fights for a life in the service of others. It is the figure of a Franklin, of an Oates, of the many names that are emblazoned on the roll of exploring fame. When will has thus risen to a superhuman might, it bows to its own mortality.



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## EXPLANATION OF THE PLATES.

## PLATE 10.

- Fig. 1. Gram-positive, sporing bacillus ( $3.6\mu \times .6\mu$ ), magn. 1,000, found in morainic mud.  
 „ 2. Gram-positive coccus ( $1.4\mu$ ), magn. 1,000, found in morainic mud.  
 „ 3. Gram-positive coccobacillus ( $1.6\mu \times 1.1\mu$ ), magn. 1,000, found in morainic mud.  
 „ 4. Gram-negative bacillus ( $2.5\mu \times .4\mu$ ), magn. 1,000, found in morainic mud.

## PLATE 11.

- Fig. 5. Gram-positive, sporing, chained bacillus ( $3.5\mu \times .8\mu$ ), magn. 1,000, found in morainic mud.  
 „ 6. Gram-positive coccus ( $1.3\mu$ ), magn. 1,000, found in morainic mud.  
 „ 7. Gram-positive coccus ( $.5\mu$ ), magn. 1,000, found in granite sand.  
 „ 8. Gram-positive coccus ( $.7\mu$ ), magn. 1,000, found in granite sand.

## PLATE 12.

- Fig. 9. Gram-positive coccus ( $1.4\mu$ ), magn. 1,000, found in dried algæ.  
 „ 10. Gram-positive bacillus ( $1.7\mu \times .3\mu$ ), magn. 1,000, found in dried algæ.  
 „ 11. Gram-positive, sporing bacillus ( $2.8\mu \times .5\mu$ ), magn. 1,000, found in dried algæ.  
 „ 12. Gram-positive, sporing, chained bacillus ( $3.5\mu \times .8\mu$ ), magn. 1,000, found in dried algæ.

## PLATE 13.

- Fig. 13. Smear of rectum, Giant Petrel.  
 „ 14. Gram-positive, sporing, chained bacillus ( $3.5\mu \times .8\mu$ ), magn. 1,000, 48 hours' culture, found in morainic mud.  
 „ 15. Film of pus from wound of Weddell Seal.  
 „ 16. Film of pus from wound of Weddell Seal with bacteria and degenerated leucocytes.

## PLATE 14.

A Ross Seal with open suppurating wounds and scars.

## PLATE 15.

Forty-eight hours' culture of an agar plate, exposed for twelve hours in the Hut.

## PLATE 16.

The terminal moraine at Cape Denison, from which the specimens of granitic sand, morainic mud, and dried algæ were procured.

## PLATE 17.

The slopes of the land-ice sheet (continental glacier) falling to the sea.

## PLATE 18.

The Hut at Cape Denison, Adelie Land—the Main Base of the Australasian Antarctic Expedition.

## PLATE 19.

The névé surface of the elevated hinterland, some 2,000 feet above sea-level and 50 miles east of the Main Base Station.

## PLATE 20.

View to the north from the ice slopes just above the cavern quarried in the glacier for magnetic observations.

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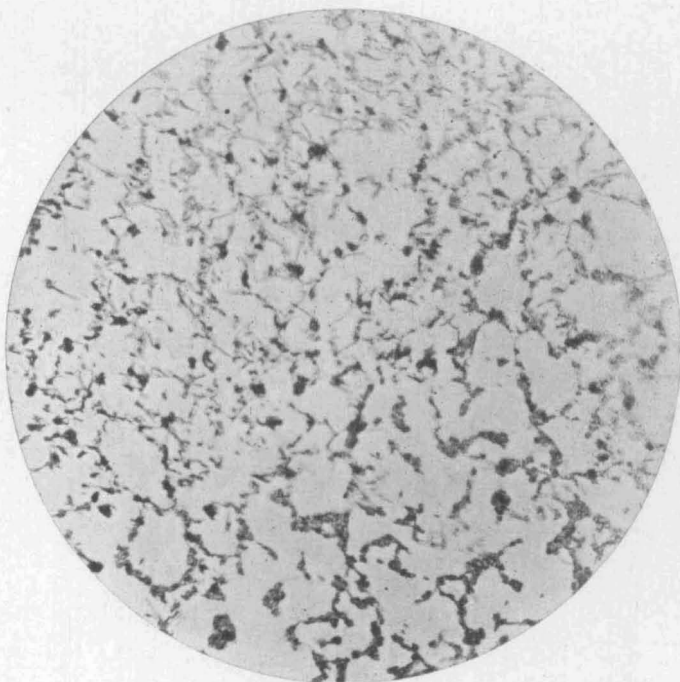
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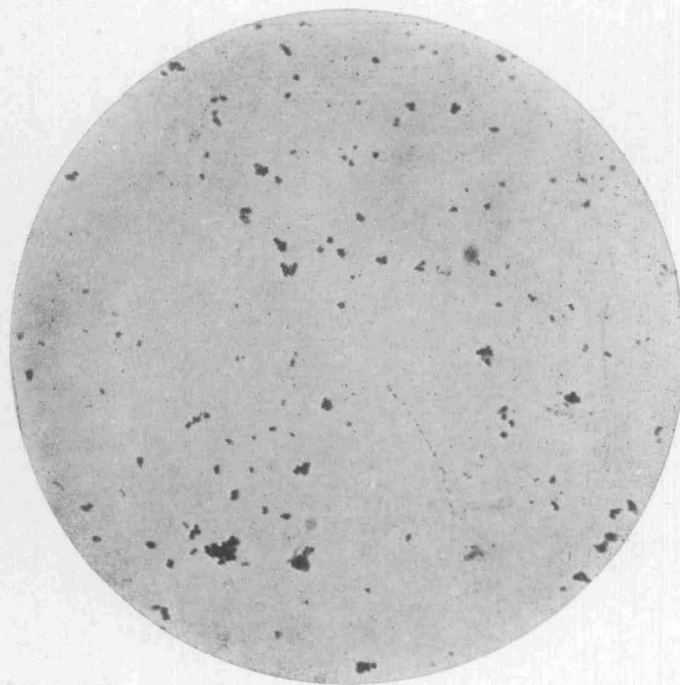
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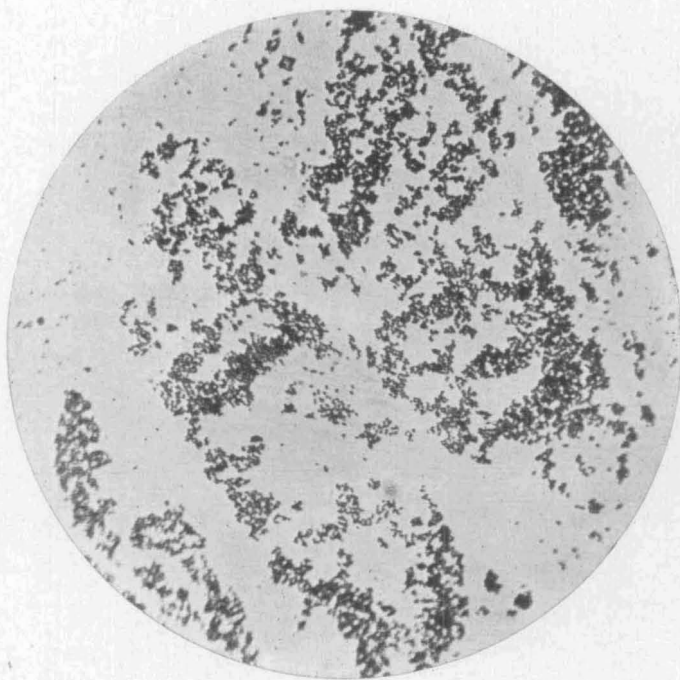
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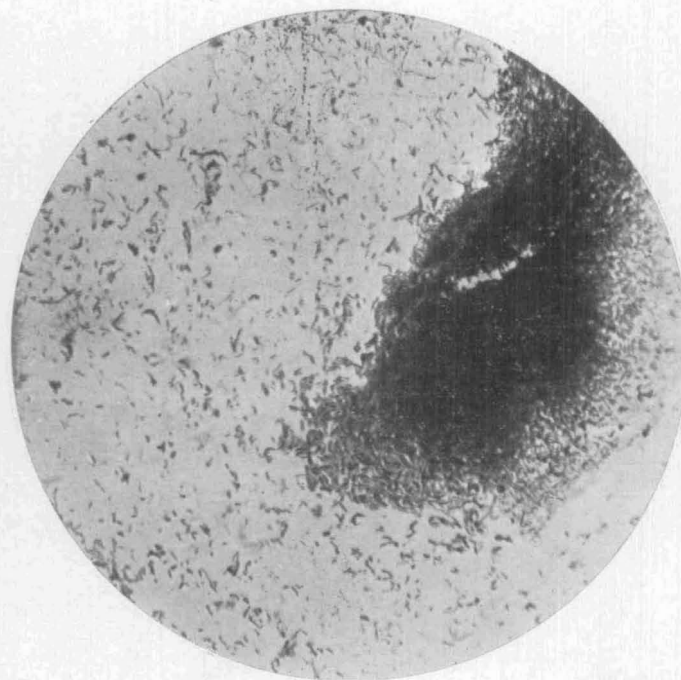
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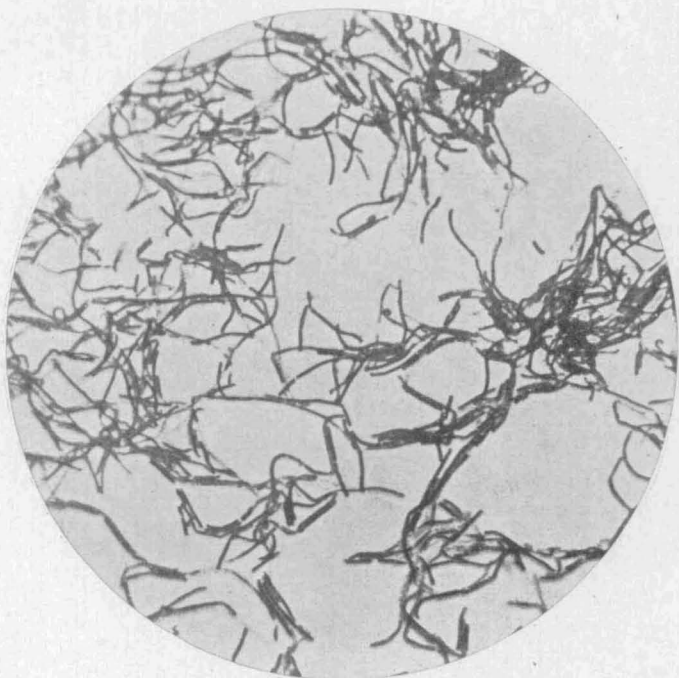


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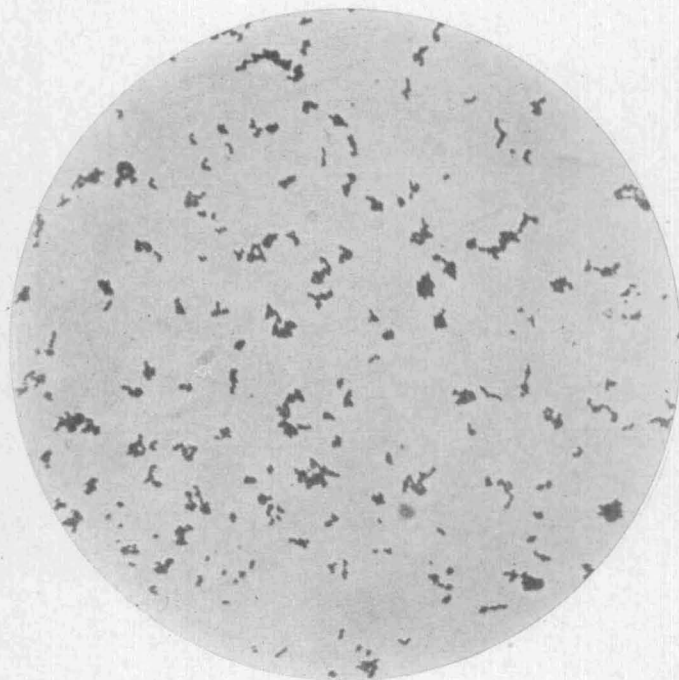




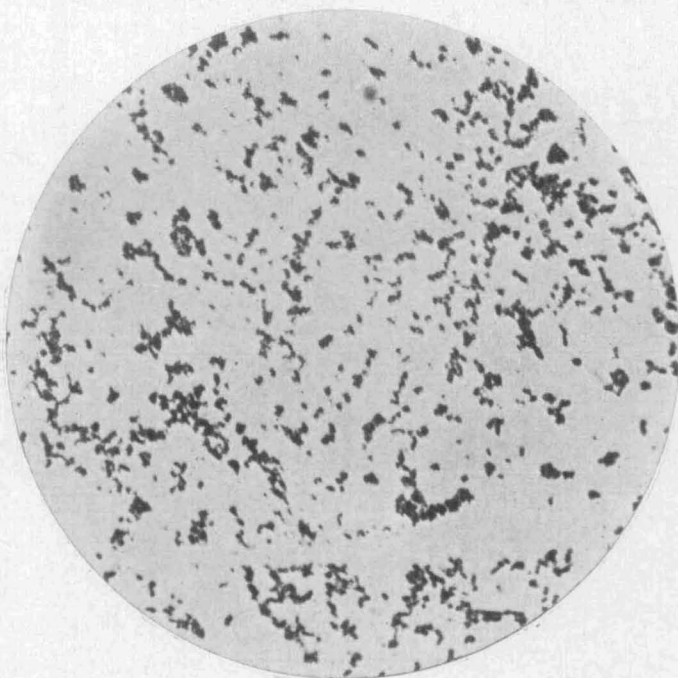
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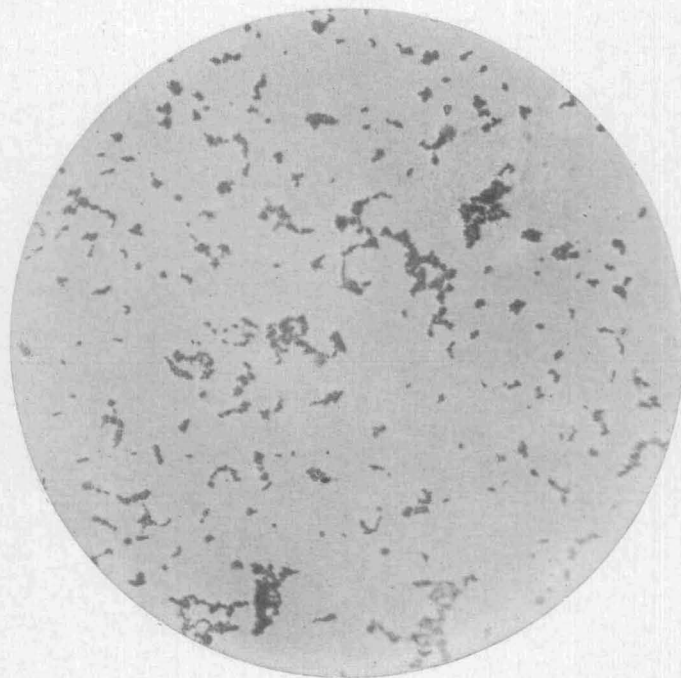
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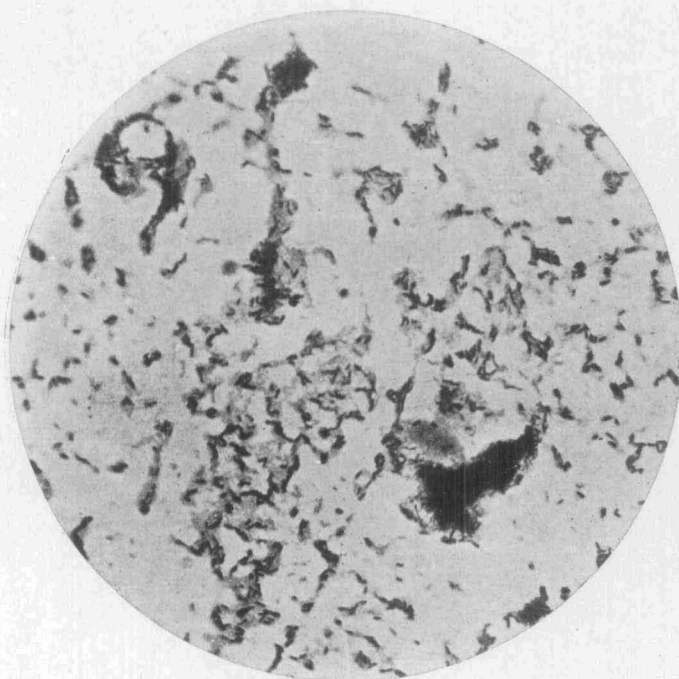


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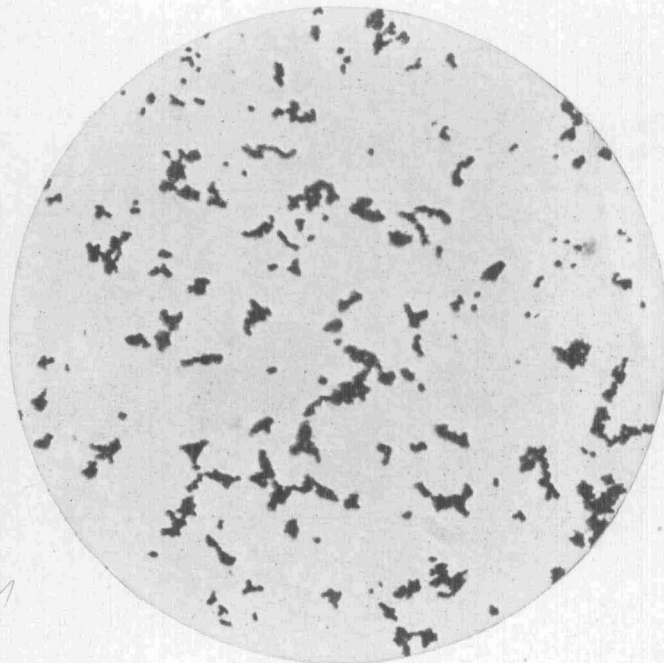




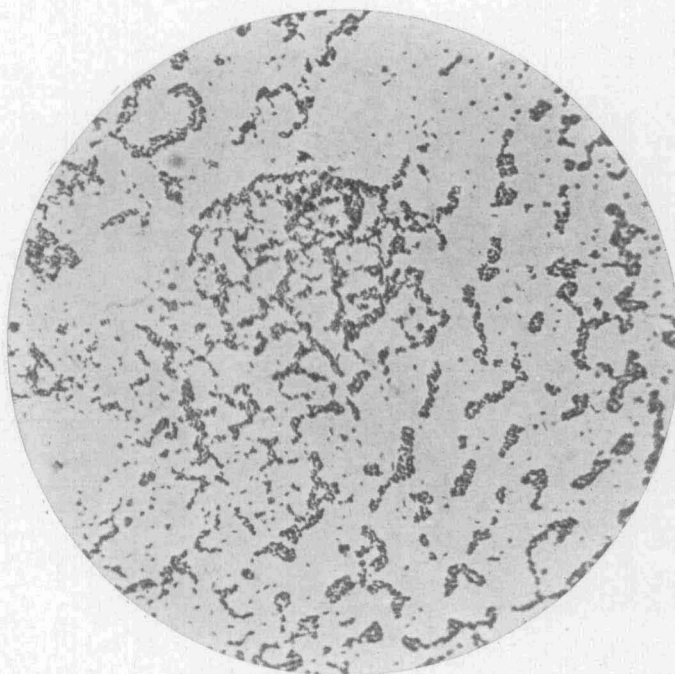
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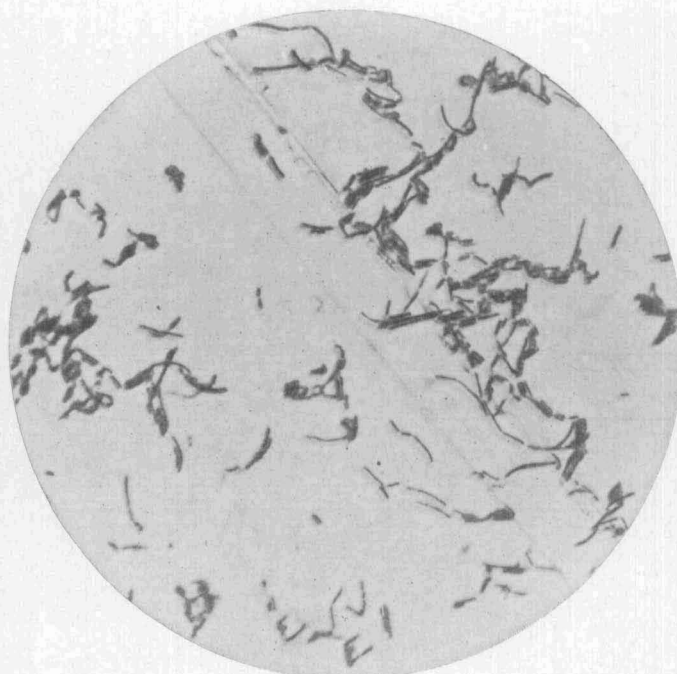
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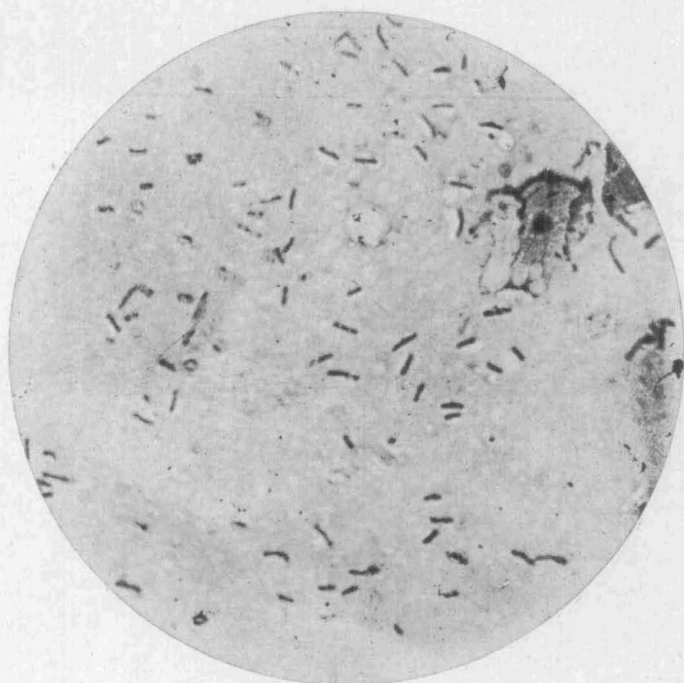
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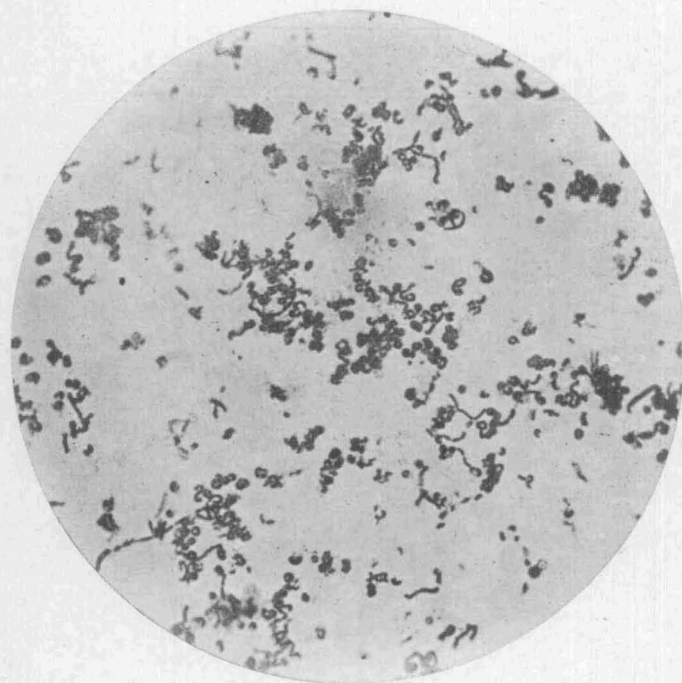
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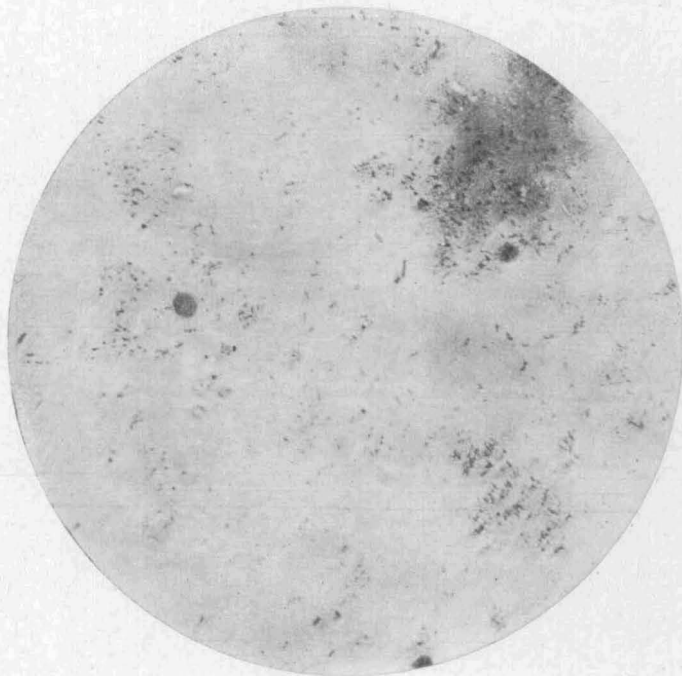
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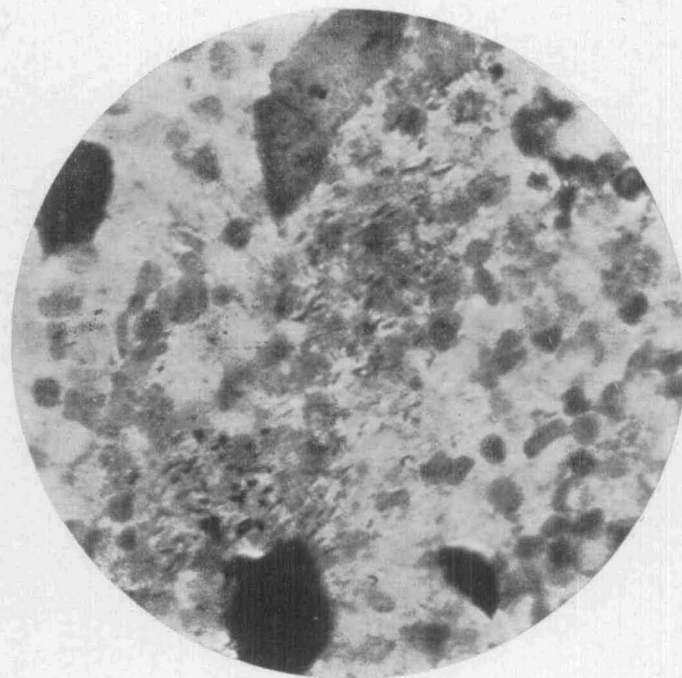
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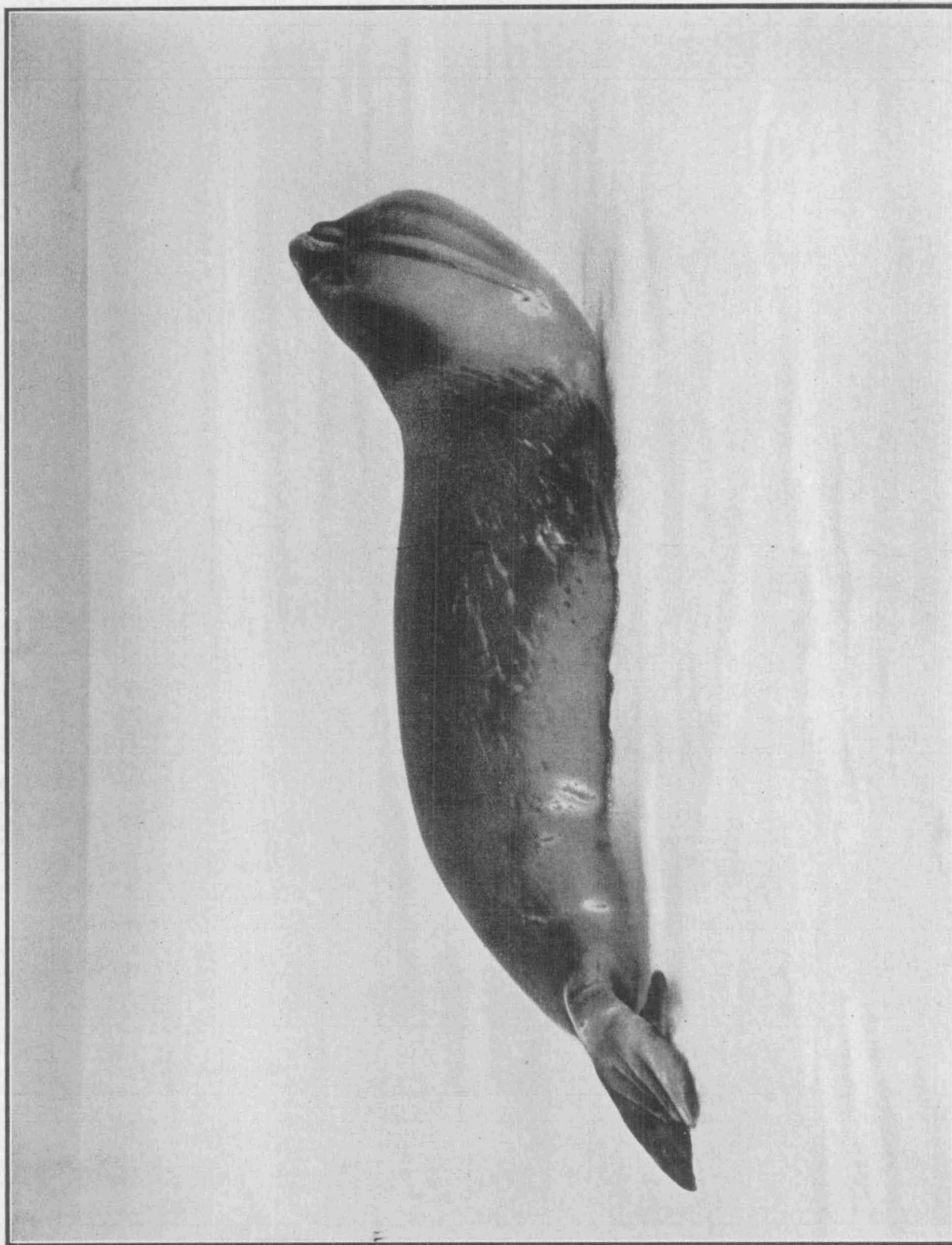
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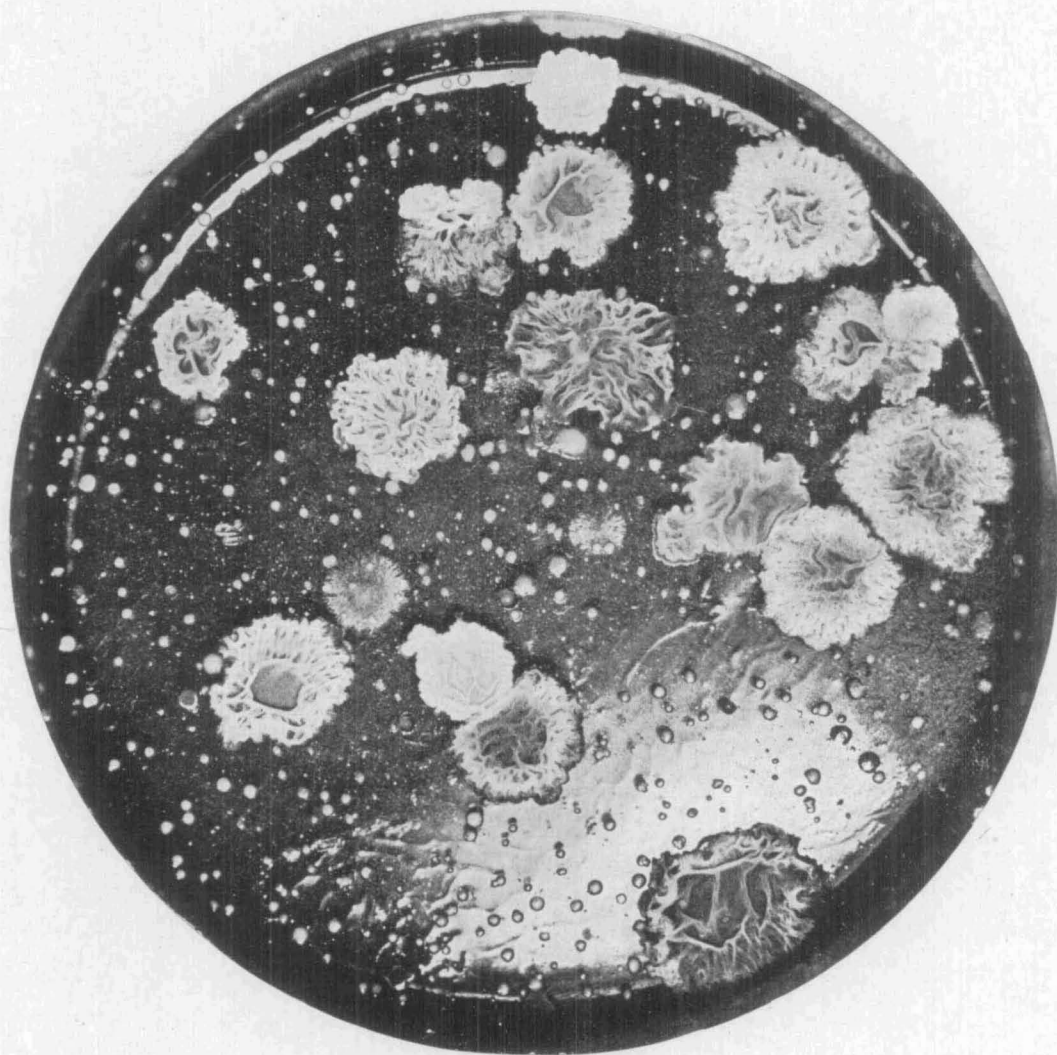
16







*J. F. Hurley, photo.*

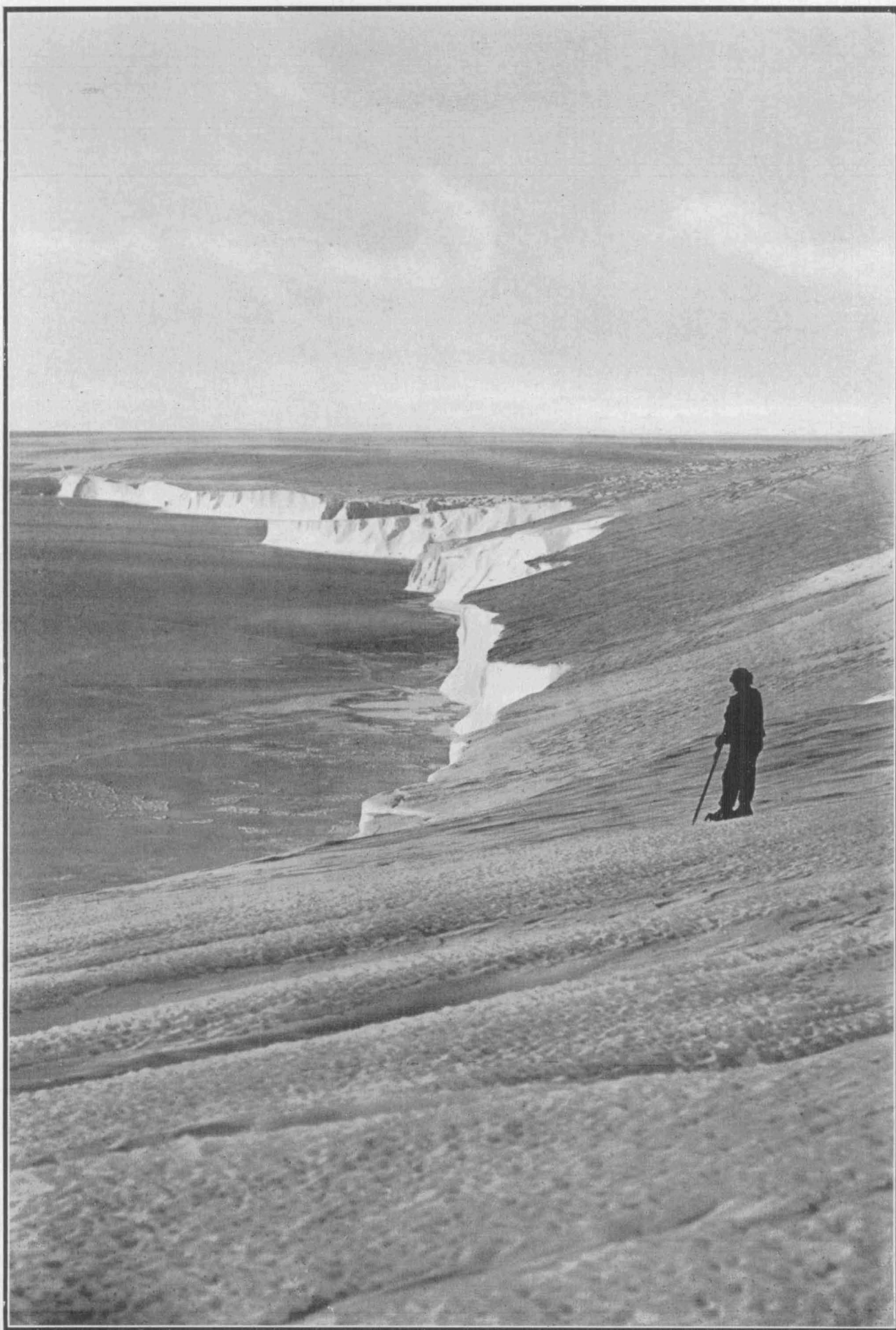


*J. F. Hurley, photo.*





*J. F. Hurley, photo.*







*J. F. Hurley, photo.*



*A. L. McLean, photo.*



