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8. The biochemical determinations were carried out by Dr. O. Boyd Houchin, assistant professor of research biochemistry and psychiatry, University of Oklahoma School of Medicine.
9. The necropsy and subsequent anatomical examinations were carried out by Professor William E. Jaques and other members of the department of pathology, University of Oklahoma School of Medicine.
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Antarctica: The Microbiology of an Unfrozen Saline Pond

Abstract. A saline pond in a region in Antarctia where other lakes and ponds are frozen remains unfrozen at the prevailing low temperatures. The ecology of the pond is unique. A distinctive aerobic microbial population, though restricted to this natural habitat, adapts to growth in artificial culture. The growth habit of these organisms, as seen in nature and in laboratory culture, indicates a possible relationship between growth at high salt concentration, at low temperatures, and in media of low organic content.

On 11 October 1961 a field reconnaissance by Navy helicopter in the south fork of the Wright Valley (longitude 161°10′E, latitude 77°34′S), Victoria Land, Antarctica, revealed an unfrozen pond, although the ambient temperature was −24°C. Wright Valley is one of several ice-free valleys in the region from which the continental icecap has receded (*1*). During the following 3 months several trips were made to the pond.

This pond, which we named Don Juan Pond, is approximately 200 m wide and 700 m long; the average depth is 11 cm (*2*). Small salt deposits occur on the periphery of the pond. The valley surrounding the pond is carved out of metasediments intruded by granites and dolerites. Overlying this valley and forming the higher ridges and peaks in the area is Beacon sandstone, also intruded by dolerites. The pond was formed by moraines blocking both ends of the valley and, at its inception, was probably 10 or more meters deep. Beach lines, now poorly defined, are present 10 m above the water level on the north side of the valley.

The influx of water into this basin is limited. During the summer two small streams drain the moraine to the west. The only other source of moisture is snow falling directly into the small catchment area immediately surrounding the pond. The snowfall averages only a few inches per year. The water is beige-to-brown in color (*3*); this is attributable in part to pyritic particles in suspension. The *p*H was 5.4 and probably remains stable, since there is little or no decay of organic matter. The water temperature followed the temperature of the ambient air; from October to December it ranged from −24° to −3.0°C, with no variation from surface to bottom. At no time during the period of study was the water frozen. The freezing point of freshly collected samples was −48° ± 1°C.

The water of Don Juan Pond was compared to the water of Barghoorn and Nichols Pond (*4*) and also to seawater (*5*) (Table 1). The specific gravity of Don Juan pond water was 1.2514, and the specific conductance was 790,000 μmho. The concentrations of dissolved solids in Don Juan pond water, in Barghoorn and Nichols pond water, and in seawater were 474,000, 132,620 and 34,480 parts per million, respectively. The salinity of Don Juan Pond was approximately 13.7 times that of seawater and 3.6 times that of Barghoorn and Nicholas Pond. Two deuterium determinations (*6*) of the pond water gave concentrations of 123.8 ppm and 124.0 ppm. By contrast, a deuterium determination on ocean water in the vicinity of McMurdo Sound gave a concentration of 160.0 ppm. These data, together with the fact that the pond is over 400 feet above sea level and 35 miles from the present coast, suggest a nonmarine origin for the water. The extraordinaryily high salt content is probably due to the weathering of the surrounding rocks, followed by increases in concentration through evaporation.

Samples of water for microbiological studies were collected in sterile flasks and returned to the Biological Laboratories at the Naval Air Facility, McMurdo Sound, Ross Island. The water was kept for several days at −22°C before laboratory study was begun. Examination of the water samples and sediments showed bacterial rods and cocci which grew in the form of colonies. Microscopic examination of bottom sediments from the pond revealed a diatom frustule, but no growing algae were present.

Table 1. Minerals in solution in antarctic waters. Composition (ppm)

Mineral	Don Juan Pond	Barghoorn and Nichols Pond	Seawater
Ca	114,000	1,130	400
Mg	1,200	4,890	1,272
Mn	<0.05		
Na	11,500	33,200	10,556
SO_4	11	16,150	2,649
Cl	212,000	58,000	18,980
CO_3	0	330	
HCO_3	49		140
NO_3	12.7		
S	0	<0.1	
Fe	23.7		
K	160		380

Live microorganisms were determined by pour plates of water samples in nutrient agar, peptone glucose-acid agar, and Proteose-peptone thioglycollate agar. The latter medium was also used to test for growth under anaerobic conditions. In addition, 100 ml aliquots were passed through Millipore filters to concentrate the organisms, and these concentrates were cultured on the several media. All plates were incubated at 20°C for 3 weeks. Three types of bacteria (*7*), *Bacillus megaterium, Micrococcus* sp., and *Corynebacterium* sp., appeared in all the media. A single yeast species (*8*), *Sporobolomyces,* which resembles *Sporidiobolus* (*9*), developed only on the sugar media. No anaerobes developed in the anaerobic plates. The fact that these isolates from an environment of remarkably high salinity and temperatures below 0°C will grow at room temperatures on artificial substrates is indicative of a high degree of adaptability. A pure culture of each bacterial isolate grew in visible clumps at temperatures ranging from 0° to 25°C when inoculated into Don Juan pond water that had been sterilized by filtration. The organisms grew more rapidly at the lower temperatures when saline pond water was used in compounding the peptone media than when

Table 2. Temperature and chemical measurements of inland water in Antarctica.

Lake	Temp. (°C)	*p*H	Conductivity (μmho)*	Chloride (mg/liter)*
Bonney (edge)	3.6	7.35	105–210	30
Vanda (edge)	2.8	7.2	112	30
Vanda (60 m)	16.0*	6.4	250,000	80,800

* Data of Armitage and House (*10*).

distilled water was used. No airborne organisms were isolated from the vicinity of the pond in repeated tests with the Andersen sampler.

To see whether the isolates from the Don Juan Pond were peculiar to the environment or whether they were also found in freshwater or less saline ponds in nearby valleys, collections were made, in sterile plastic bottles, from meltwater on the edges of Lake Bonney, in Taylor Valley, and Lake Vanda, in Wright Valley—lakes which are permanently covered with ice. Samples were also obtained from the 60-m level of Lake Vanda. The temperatures and results of chemical analyses of these lakes are shown in Table 2 (*10*).

From standard dilution plates of each meltwater sample prepared with plain nutrient and peptone glucose-acid agars, incubated at 22°C, four types of bacteria were isolated. These were Gram-negative and Gram-positive nonmotile rods and Gram-positive cocci. A yeast growth form was obtained from Lake Vanda; the yeast *Sporobolomyces* was isolated only from Lake Bonney. No organisms were isolated from the 60 m level of Lake Vanda. None of the organisms from Lake Vanda and Lake Bonney appear to be the same as those isolated from the saline Don Juan Pond.

The Don Juan Pond provides an unusual ecological picture. A distinctive relative concentration of ions results in a high salt content. The aerobic microbial population is restricted in natural habitat and is not found in nearby waters. Yet in the laboratory these microbes can be cultured over a wide range of salt concentrations, temperatures, and nutrients. Their habit of growing in colonies, as observed in direct examination of the natural habitat, is retained in laboratory culture in broth of varying salinity and incubation temperature. Our preliminary studies indicate a possible relationship between high salt concentration and the ability to grow at low temperatures and in an environment of low organic content (*11*).

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Hippocampal Ablation and Passive Avoidance

Abstract. Six rats with bilateral ablation of the hippocampus and six rats with extensive destruction of the neocortex were trained to enter a small compartment, while hungry, for a food reward. After 35 trials, spread over 4 days of training, the animals were given a shock while they were eating in the goal box. After the shock the rats with cortical lesions would not enter the goal compartment on the remainder of the trials given on the same day and only gradually began to re-enter over the next 2 days. The effect of the shock on the subjects with hippocampal ablation was slight and transient, suggesting that the ability to make passive avoidance responses was impaired.

An anatomical dissociation of the abilities necessary to acquire active and passive avoidance responses has been indicated by recent work of McCleary (*1*). Cats with lesions beneath the genu of the corpus callosum had difficulty in acquiring a passive avoidance response, but their ability to acquire an active avoidance response seemed unimpaired. Animals with lesions of the cingulate cortex showed just the opposite deficit. Since rats with radical bilateral lesions involving from 50 to 90 percent of the hippocampus have no difficulty in acquiring an active avoidance response (*2*), we designed a study to determine whether such lesions would interfere with their acquiring a passive avoidance response.

Twelve rats of the Sprague-Dawley strain were used as subjects. When the rats were about 90 days old, radical bilateral hippocampal ablation was carried out in six of the animals and extensive neocortical lesions were made in the other six. The surgical techniques used were similar to those described by Isaacson, Douglas, and Moore (*2*). About 2 weeks later the animals were put on a 23-hour food deprivation schedule; this was maintained throughout the experiment. During the experiment the body weights of the subjects were maintained at 85 percent of the preoperative weights. All the subjects were given experience, prior to the experiment, in learning to seek food rewards in a maze. The experiment was begun when the animals were about 120 days old.

The apparatus consisted of two compartments separated by a guillotine-type door. The larger compartment had unpainted wood walls and floor and a lid of transparent plastic. The floor area of this compartment was 12 by 18 inches; the height was 10 inches. The smaller compartment, connected to the larger compartment by a passageway, had wooden walls, a copper grid floor, and a wire mesh top. The floor area of this smaller compartment was 6 by 4 inches; the height was 4 inches. A small copper tray about 1 by ¾ by ¼ inch was attached to that end of the smaller compartment farthest away from the passageway. Food rewards in the form of wet mash made from food powder were given in this tray during training.

The animals were allowed to explore the apparatus for half an hour on the day prior to the commencement of training. Every day at the start of the training period the subject was placed in the larger compartment for several minutes while the door leading to the smaller compartment was closed. Then the door was opened and the animal could enter the smaller compartment and receive, in the feeding tray, a small amount of the wet mash. While the rat was eating in the smaller compartment some of the food was placed in the larger compartment as a lure to induce it to leave the smaller compartment and return to the larger one. As the animal re-entered the larger compartment, the guillotine door was closed. The amount of food given the animal in the smaller compartment was always greater than that given it in the larger compartment. Between 20 and 60 seconds after the animal had eaten