GASTROINTESTINAL MICROFLORA OF ANTARCTIC BIRDS[1]

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Most of the information on the gastrointestinal microflora of antarctic birds was obtained during the great decade of South-Polar exploration following the turn of the century. The report of Levin (1899) that bacteria could not be found in the intestines of arctic birds at Spitzbergen prompted similar studies by the naturalists and ship's physicians on the Antarctic expeditions. Ekelöf (1908) on the 1901–1903 Swedish South Polar Expedition cultured the intestinal contents of antarctic birds at Snow Hill Island with negative findings except for two skua gulls. Gazert (1912) on the 1902–1903 German South Polar Expedition to Kerguelen and Heard Islands cultured stomach and intestinal contents of birds and incubated both aerobically and anaerobically. No bacteria were obtained from the petrels and only one tern and one Adélie penguin yielded bacterial growth. Harvey Pirie (1912), during the 1902–1904 Scottish National Antarctic Expedition to Laurie Island and the Weddell Sea, made agar and gelatin stab cultures of stomach and intestinal material and obtained bacterial growth from 12 of the 21 birds cultured. Dr. Charcot, who led the 1903–1905 French Antarctic Expedition to the western side of Palmer Peninsula, examined intestinal material from gulls, petrels, and penguins and found many types, although in smaller numbers than in temperate regions. Tsiklinsky (1908) identified a few species from Charcot's cultures. McLean (1919) on the 1911–1914 Australasian Antarctic Expedition reported the absence of bacteria in 6 of 14 antarctic birds at Commonwealth Bay. These investigations between 1901 and 1914 mainly reported by species those birds that contained bacteria and those that were "bacteriologically sterile." Bunt (1955) during the 1951–1952 Australian Antarctic Expedition to Macquarie Island repeated the classical qualitative approach of the earlier workers and reported that 2 of the 18 birds cultured were "bacteriologically sterile."

During the 1957–1958 Argentine Antarctic Expedition through the South American Quadrant of Antarctica, bacteriological studies were conducted in a temporary bacteriology laboratory aboard the icebreaker *ARA General San Martin* (Sieburth, 1958). Birds were collected during the numerous opportunities to go ashore by landing craft and helicopter. The primary purpose of this study was to examine quantitatively gastrointestinal material in an attempt to explain the reports of "bacteriologically sterile" antarctic birds. Data are presented which show the estimated bacterial population, predominant cultivable bacterial types, and the ability of gastrointestinal material to enhance or inhibit bacterial growth. Antibacterial dietary factors are described which apparently modify the gastrointestinal flora and may explain the earlier reports of "bacteriologically sterile" antarctic birds.

MATERIALS AND METHODS

Instruments, special materials, and preweighed dyes and reagents in dry form were the only materials taken to Argentina. All glassware and bulk chemicals were graciously supplied by the Hydrographic Service of the Argentine Navy. With the cooperation of Dr. R. Copes, the ship's physician, a laboratory was set up in a small pharmacy, that had a small working surface, refrigerator, sink, balance, and microscope. A hot air oven, incubator, and autoclave were available in the adjacent infirmary. All supplies and materials had to be secure at all times due to the 45 degree 8 second lateral roll cycle of the icebreaker in open sea, which was extremely

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hard on glassware and the maintenance of bacterial cultures.

The flying birds were shot and the penguins were sacrificed by cervical dislocation. The birds were opened as aseptically as possible, the entire gastrointestinal tract was removed and samples were aseptically taken from either the proventriculus or ventriculus, central intestine, and terminal intestine. One g samples of the ingesta were triturated with Eugon broth (BBL) to yield 10 per cent suspensions for bacterial enumeration and Gram stained smears for microscopic examination for the presence of bacteria and predominant bacterial forms. Since poured petri plates readily became contaminated with mold, all work was done in tubed media. Serial decimal dilutions of the gastrointestinal suspensions were made in broth and 0.1-ml amounts of each dilution were smeared on two long Eugon agar slants (total aerobe and anaerobe counts) and one long Tergitol-7 agar slant (Difco) for the coliform count. One ml of each dilution was used to inoculate a tube of SF broth (Difco) for enteric streptococci. Anaerobic conditions were obtained by the simple but effective method of Parker (1955). All cultures were incubated at 38 C for 24 hr since longer incubation did not appreciably alter the counts and incubation and storage space were limited. Representative colonies from the highest dilutions having growth were isolated in order to study the predominant bacterial types. The bacterial isolates that remained viable at the conclusion of the voyage were studied according to the *Manual of Microbiological Methods* (Pelczar and Conn, 1957) and the cultures other than *Enterobacteriaceae* were identified according to *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1957). The isolates of *Enterobacteriaceae* were kindly identified by Dr. W. H. Ewing, Communicable Disease Center, Chamblee, Georgia.

In order to detect the presence of bacterial inhibiting and enhancing substances in the nonsterile gastrointestinal material, a procedure was developed that utilized Millipore filter membranes, pads, and plastic dishes. The broth suspensions (approximately 1.6 ml) of the gastrointestinal material were used to soak the pad which was covered by a sterile hydrosol assay membrane that provided a barrier from the contaminated material and a sterile surface upon which to streak the test bacteria. The test organisms included a typical strain of *Escherichia coli*, two delayed lactose fermenting strains of *E. coli*, *Micrococcus ureae*, and a diphtheroid-like gram-positive rod which were all isolated from skua gulls. The growth of the test organisms on the broth control was compared with that on the test material. This procedure was later modified to test the antibacterial activity of blood serum.

RESULTS

The birds were obtained between December 5, 1957, and February 5, 1958, at the following locations: Ushuaia, Tierra de Fuego Island (1); Half Moon Island, South Shetland Islands (3, 4, 5, 14); Almirante Brown Base (15) and Hope Bay (8, 11, 12, 20) on Palmer Peninsula; Laurie Island, South Orkney Islands (21, 28); Southern Thule Island (23) and Zavodovski Island (26, 27) in the South Sandwich Islands; and General Belgrano Base (24) and Halley Bay (25) on the Weddell Sea shelf ice.

During the early part of this study the intestinal contents from the anterior, central, and posterior segments were pooled. When great individual differences were observed between birds, each bird was studied in greater detail, separate observations being made on the proventriculus, ventriculus, and central and terminal areas of the intestine. Rather than study as many species as possible, a few species were studied according to their feeding habits. As examples of scavengers and predators the skua gulls, sheathbills, and one giant fulmar were studied. The penguins were studied as examples of birds feeding on marine animals. The birds were identified according to Murphy (1936).

Gastrointestinal flora of scavenging and predatory birds. The results on eight flying birds with scavenging and predatory feeding habits are given in table 1. The cultivable bacterial population is expressed as the log of the number of organisms per g of wet material. The effect of the gastrointestinal material on the growth of the test organisms is expressed as relative inhibition (−) or growth enhancement (+). The predominant bacterial types found in each gastrointestinal segment are shown. All four skua gulls had an aerobic, facultatively anaerobic flora. Rapid lactose fermenting coliforms were present in all four birds although delayed lactose strains were more numerous. The enteric group

TABLE 1

Estimated bacterial population, bacterial activity, and predominant bacterial types in the gastrointestinal material of antarctic flying birds

Avian Species	Bird No.	Gastrointestinal Segment	Estimated Bacterial Population				Effect of Gastrointestinal Material on Growth of			Predominant Bacterial Isolates
			Aerobes			Anaerobes	Gram-positive	*Escherichia coli* (atypical)	*Escherichia coli* (typical)	
			Total	Coliform	Enterococci					
Skua gull	1	Intestine	3.5×10^5	3.0×10^3	$<10^1$	3.5×10^5	—	—	—	*Achromobacter eurydice*, *Micrococcus ureae*. Bethesda Group, Diphtheroid-like.
	4	Intestine	2.0×10^6	6.4×10^5	$<10^1$	5.0×10^5	—	—	—	*E. coli* (atypical)
	8	Intestine	5.5×10^3	2.5×10^3	$<10^1$	3.5×10^3	—	—	—	*E. coli* (atypical)
	26	Ventriculus	2.5×10^6	1.0×10^6	$<10^1$	6.1×10^6	−9	0	−6	*E. coli* (atypical and typical)
		Central intestine	1.5×10^7	1.0×10^7	$<10^1$	1.2×10^7	−9	−3	+6	*E. coli* (typical)
		Terminal intestine	1.1×10^7	1.1×10^7	$<10^1$	4.8×10^7	−9	−6	0	*E. coli* (typical) and *Streptococci sp.*
Sheathbill	3	Intestine	6.8×10^5	7.3×10^3	$<10^1$	2.1×10^6	—	—	—	*E. coli* (atypical)
	11	Intestine	$<10^2$	$<10^2$	$<10^1$	8.0×10^2	—	—	—	Anaerobic, gram-positive rods
	28	Proventriculus	3.1×10^4	$2.4. \times 10^4$	$<10^1$	1.5×10^5	−3	+6	+6	*E. coli* (atypical), Diphtheroid-like
		Central intestine	4.8×10^6	3.3×10^6	$<10^1$	8.2×10^6	−3	0	0	*E. coli* (typical)
		Terminal intestine	1.1×10^7	9.5×10^6	$<10^1$	4.0×10^7	−9	−9	−9	*Aerobacter-Hafnia* sp., Diphtheroid-like
Giant fulmar		Proventriculus	$<10^2$	$<10^2$	$<10^1$	$<10^2$	−9	−9	−9	None
	27	Central intestine	$<10^2$	$<10^2$	$<10^1$	$<10^2$	−9	+9	+6	None
		Terminal intestine	2.0×10^2	$<10^2$	$<10^1$	8.0×10^4	−9	+9	+6	"Anaerobic" streptococci

of streptococci were not detected in these or any of the other birds studied although other streptococcal species were observed. The isolates from bird no. 1 which was scavenging in the harbor at Ushuaia, were the most diversified of any of the birds studied. The simple flora of skua gulls 4 and 8 from the more isolated bases consisted of delayed lactose fermenting strains (atypical) of *E. coli* with typical strains in smaller numbers. The gastrointestinal material of bird 26, which was preying on penguin chicks on uninhabited Zavadovski Island, was quite inhibitory for the gram-positive test organisms and gram-positive organisms were only found in the terminal portion of the intestine. The ventriculus contents were inhibitory for typical *E. coli* but not for atypical strains which were the most numerous. The intestinal material was inhibitory for the atypical *E. coli* types which were not detected, but not for the typical types which were the most numerous.

Sheathbill no. 3 was scavenging on seal carcasses near the sledge dogs at Half Moon Island. The flora of this bird was also aerobic and facultatively anaerobic. Although rapid lactose fermenting strains of *E. coli* were present, atypical strains constituted the predominant flora. Bird 11, which was obtained in the Adélie penguin rookery at Hope Bay and was presumably feeding in the intertidal zone, was devoid of a detectable aerobic flora. However, a small anaerobic flora of gram-positive rods was present. The third sheathbill, no. 28, had been feeding on a mixture of birds eggs, marine forms, and refuse from the Laurie Island weather station. The flora was aerobic and facultatively anaerobic. Although all segments were inhibitory for gram-positive organisms, diphtheroid-like organisms were

present in both the proventriculus and terminal intestine. The contents of the proventriculus and central intestine were noninhibitory for *E. coli* which was the predominant organism in these segments. The material in the terminal intestine was very inhibitory to both typical and atypical *E. coli* and an *Aerobacter-Hafnia* species was the predominant gram-negative organism present.

The only giant fulmar studied was bird 27, which like skua no. 26, was obtained on Zavodovski Island and had been feeding on penguin chicks. The proventriculus contents were very inhibitory for all the test bacteria and no cultivable bacteria were observed. Although the contents of the central intestine were stimulatory for *E. coli*, this material also failed to yield detectable bacterial growth. The contents in all three segments were very inhibitory for gram-positive bacteria. However, Gram stained smears indicated that an apparently homogeneous population of large streptococci was present in all segments. This organism was cultivated poorly aerobically and better anaerobically only in material from the terminal intestine.

Gastrointestinal flora of penguins. The results on eight penguins feeding on marine forms are given in table 2. All the penguins except the emperor penguin chick no. 25 were feeding on the lobster krill *Euphausia superba* and were

TABLE 2

Estimated bacterial population, bacterial activity, and predominant bacterial types in the gastrointestinal material of antarctic penguins

Avian Species	Bird No.	Gastrointestinal Segment	Estimated Bacterial Population				Effect of Gastrointestinal Material on growth of			Predominant Bacterial Isolates
			Aerobes			Anaerobes	Gram-positive	*Escherichia coli* (atypical)	*Escherichia coli* (typical)	
			Total	Coliforms	Enterococci					
Adélie penguin	5	Intestine	$<10^2$	$<10^2$	$<10^1$	2.5×10^4	—	—	—	Anaerobic, gram-positive rods
	12	Intestine	$<10^2$	$<10^2$	$<10^1$	1.2×10^3	—	—	—	Anaerobic, gram-positive rods
	20	Proventriculus	—	—	—	—	−9	+3	+6	
		Ventriculus	2.0×10^5	$<10^2$	$<10^1$	4.8×10^5	0	−1.5	−3*	
		Central intestine	1.2×10^3	$<10^2$	$<10^1$	1.3×10^6	+7	+6	+6	Fastidious gram-negative rods
		Terminal intestine	1.6×10^5	$<10^2$	$<10^1$	2.2×10^6	0	0	0	
Gentoo penguin	14	Ventriculus	$<10^2$	$<10^2$	$<10^1$	2.0×10^2	—	—	—	
		Central intestine	4.3×10^4	$<10^2$†	$<10^1$	1.0×10^5	—	—	—	*E. coli* (atypical)
		Terminal intestine	1.3×10^6	$<10^2$	$<10^1$	2.1×10^6	—	—	—	
	15	Ventriculus	$<10^2$	$<10^2$	$<10^1$	$<10^2$	—	−9	—	None
		Central intestine	4.9×10^5	$<10^2$†	$<10^1$	3.1×10^5	—	+7	—	Providence Group
		Terminal intestine	2.4×10^7	$<10^2$	$<10^1$	3.7×10^7	—	0	—	
Ringed penguin	21	Proventriculus	3.5×10^3	$<10^2$	$<10^1$	3.6×10^3	+9	+6	+6	
		Ventriculus	7.0×10^3	$<10^2$	$<10^1$	1.9×10^4	+9	+6	+6	
		Central intestine	1.3×10^4	$<10^2$	$<10^1$	5.5×10^4	+9	+6	+6	Fastidious gram-negative rods, *Streptococcus equinus*
		Terminal intestine	2.6×10^4	$<10^2$	$<10^1$	5.5×10^4	+3	+3	+6	
	23	Proventriculus	2.2×10^6	$<10^2$	$<10^1$	7.5×10^6	+4	+6	+6	
		Ventriculus	1.0×10^3	$<10^2$	$<10^1$	1.3×10^7	+2	0	0	
		Central intestine	3.5×10^3	$<10^2$	$<10^1$	1.5×10^6	−2	+6	0	Fastidious gram-negative rods, *S. equinus*
		Terminal intestine	4.0×10^5	$<10^2$	$<10^1$	2.6×10^6	−9	+6	+3	
Emperor penguin	25	Ventriculus	1.2×10^4	1.0×10^3	$<10^1$	1.4×10^4	−6	−9	0	
		Central intestine	1.8×10^5	2.4×10^4	$<10^1$	8.0×10^4	−6	0	0	Providence group, *E. coli* (typical)
		Terminal intestine	9.2×10^7	2.1×10^7	$<10^1$	1.2×10^8	−9	−9	−6	

* Inhibitory to own aerobic flora.

† No lactose fermenting coliforms detected, lactose nonfermenting organisms, equal or higher than total aerobic count.

devoid of typical strains of *E. coli*. As in the flying birds, no streptococci of the enteric group were detected. Adélie penguins 5 and 12 were free of a detectable aerobic flora. Both of these penguins had a small but detectable anaerobic flora which consisted of pleomorphic gram-positive rods that grew poorly on subculture and were soon lost. Adélie penguin no. 20 had a flora in all segments which grew more luxuriantly under anaerobic conditions. These fastidious lactose nonfermenting gram-negative rods soon died out on subculture. The material in the proventriculus was very inhibitory for gram-positive bacteria and that in the ventriculus was very inhibitory for its own aerobic flora. These observations might explain the low aerobic population in the central intestine and the absence of gram-positive bacteria. Despite the growth enhancing properties of the contents of the central intestine for the test bacteria, the bacterial population did not increase one log place throughout the gastrointestinal tract.

The gentoo penguins (birds 14 and 15) came from different areas, but shared a number of characteristics. They were heavily infested with cestodes and the epithelial lining of the proventriculus and ventriculus was discolored green by the phytoplankton in the stomach of the lobster krill upon which the birds were feeding. Although the ventriculus contents had a negligible flora, Gram stained preparations revealed an abundant gram-negative coccobacillary flora which failed to grow upon repeated cultivation. Rapid lactose fermenting coliforms were not detected, however, the Tergitol-7 slants contained a population equal to or greater than that on the Eugon agar medium. In bird 14, the flora consisted of a lactose nonfermenting *E. coli*. The predominant flora of bird 15 belonged in the Providence Group. The ventriculus contents of this bird, which inhibited four atypical *E. coli* strains, were free of a cultural flora while the contents of the central intestine which were growth enhancing yielded a bacterial flora.

The ringed penguins (birds 21 and 23) also came from different areas and shared a number of characteristics. There was both an aerobic and a facultatively anaerobic flora in each of the four segments studied. Neither of the birds contained detectable numbers of coliforms. The predominant flora of both birds consisted of a fastidious lactose nonfermenting gram-negative rod and *Streptococcus equinus*. These penguins were feeding on lobster krill that apparently had not been feeding recently on phytoplankton. The gastrointestinal material was growth enhancing rather than inhibitory.

The emperor penguin chick, no. 25, was the only penguin studied in detail which was not feeding on lobster krill. The proventriculus contained a fluid with black particulate matter, approximately 200 g of pebbles, and 10 pairs of cephalopod beaks. The aerobic and anaerobic populations were similar and a cultivable flora was present in all segments despite the inhibitory activity of the proventriculus contents which were highly acidic (pH 1.35). All segments were quite inhibitory for the gram-positive test organisms. The predominant organisms belonged to the providence group. Typical strains of *E. coli* were also numerous and were the only rapid lactose fermenting strains found in any of the penguins studied. Emperor penguins can go for long periods without eating (at least 40 days). In order to determine if a bacterial flora persists in such a starved bird, an adult emperor penguin (no. 24) which had been held captive at the General Belgrano Base without food for 15 days was briefly studied. Oral and rectal swabs indicated an abundant flora at both ends of the gastrointestinal tract. Diphtheroid-like and klebsiella-like organisms were isolated from the rectal cultures.

Biochemical activities of the predominant enterobacterial types. Of the 16 birds studied in detail, four had an obligately anaerobic flora. Three birds had a flora of fastidious gram-negative rods with *S. equinus* being present in lower numbers in two of these birds. The remaining nine birds had an aerobic, facultatively anaerobic flora in which members of the family *Enterobacteriaceae* were the predominant types. The biochemical characteristics of these enterobacterial types are given in table 3.

All nine birds had delayed or negative lactose fermenting types instead of, or in addition to, the rapid lactose fermenting types. Although rapid lactose fermenting coliforms were present in six of the eight flying birds, typical strains of *E. coli* only predominated in skua gull 26 and sheathbill 28. Only one of the eight penguins (no. 25) had typical strains of *E. coli*.

The atypical *E. coli* strains showed different carbohydrate fermentation patterns. Organisms

TABLE 3

Biochemical activities of the enterobacterial types that constituted the predominant aerobic intestinal microflora of 10 antarctic birds

Enterobacterial Type	O Antigen Group	Dextrose	Lactose	Sucrose	Maltose	Mannose	Indole	Methyl Red	Voges-Proskauer	Citrate	Nitrate Reduction	Gelatin Liquefaction	Urease	Motility	H_2S Production	Source of Cultures
Escherichia coli (typical)	OXI, 88	AG	AG	−	AG	AG	+	+	−	−	+	−	−	+	−	Skua gull (26), Sheathbill (28)
	88	A	A	−	A	−	+	+	−	−	+	−	−	−	−	Skua gull (26), Sheathbill (28)
Escherichia coli (atypical)	84	Ag	a	−	−	Ag	+	+	−	−	+	−	−	+	−	Sheathbill (3)
	98	AG	a	−	AG	AG	+	+	−	−	+	−	−	+	−	Skua gull (4, 8)
	11	AG	−	AG	AG	AG	+	+	−	−	+	−	−	+	+	Gentoo penguin (14)
Providence group	−	Ag	−	−	Ag	−	+	+	−	−	+	−	−	+	+	Gentoo penguin (15) Emperor penguin (25)
Bethesda group	8	AG	−	−	AG	AG	−	+	−	+	+	−	−	+	+	Skua gull (1)
Aerobacter-Hafnia species	−	Ag	−	−	Ag	Ag	−	+	−	−	−	−	−	+	−	Sheathbill (28)
Klebsiella-like	−	AG	AG	AG	AG	AG	+	+	−	+	+	−	−	+	−	Emperor penguin (24)

A = Acid, a = delayed acid; G = Gas; g = small amount of gas; + = positive reaction; and − = negative reaction. All carbohydrates were observed for 14 days.

from skua gull 26 and sheathbill 28 failed to produce gas or attack mannitol. The strain from sheathbill 3 attacked only dextrose and mannitol rapidly. The strains from skua gulls 4 and 8 were typical except for delayed action on lactose. The strains from gentoo penguin 14 failed to attack lactose in two to three weeks and produced hydrogen sulfide.

The noncoli enterobacterial isolates fell into four other groups. The gentoo penguin 15 and emperor penguin 25 which were obtained from widely separated areas had a predominant flora of Providence Group organisms. Skua gull 1 living close to human habitation contained an organism of the Bethesda group. Sheathbill 28, in addition to typical and atypical strains of *E. coli* also contained an *Aerobacter-Hafnia* species. A klebsiella-like organism was obtained from emperor penguin 24.

Antibacterial food materials. The results on the gastrointestinal microflora indicated that the diet of both the predatory birds and the penguins contained antibacterial materials which were apparently influencing the gastrointestinal microflora. In an attempt to determine if the antibacterial activity of blood serum could account for the inhibitory activity of gastric material in the predatory birds, 12 sera were tested for their gram-negative antibacterial activity. Serum dilutions of 1:20, 1:40, 1:80, and 1:160 in Eugon broth were used to soak the Millipore filter pads. One typical and two atypical strains of *E. coli* were streaked on hydrosol assay Millipore membranes placed on the nutrient pads. Growth was compared with that on the broth controls and the results are given in figure 1. All 12 sera were inhibitory at a 1:20 dilution, 10 at 1:40, 7 at 1:80, and 5 inhibited at least as high as a 1:160 dilution. Although the gram-positive test organisms were not used in this trial, other tests indicated that penguin serum was active against gram-positive bacteria.

The proventriculus and ventriculus contents of the penguins feeding on lobster krill had a variable activity against the test organisms. When the lining of these organs was discolored with phytoplankton, presumably from the gastric contents of the krill, this activity was marked and appeared to modify the gastrointestinal flora. In an attempt to verify this observation, a number of live adult krill (*Euphausia superba*), about 2 in in length, were obtained at Half Moon Island. The krill were dissected and the different segments were stored in crushed ice for eight days until the *San Martin* returned. The effect of 10 per cent suspensions of the krill segments on bacterial growth is shown in table 4. Only the stomach contents of the krill were active. This material which consisted of phytoplankton and was a bright green in color completely inhibited the gram-positive test bacteria. During the trip into the Weddell Sea, a number of immature krill about ¼ in long were caught in the plankton net. Since they also had green

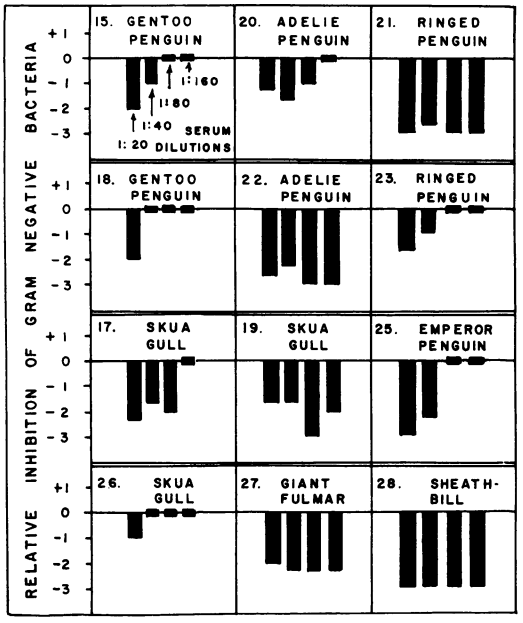

Figure 1. The inhibitory effect of blood serum from antarctic birds on gram-negative bacteria.

TABLE 4

Effect of adult lobster krill (Euphausia superba) on bacterial growth

Krill Segment*	Growth of Test Organisms†				
	Micrococcus ureae	Diphtheroid-like	*Escherichia coli* (atypical)		*E. coli* (typical)
Broth control..	2+	+	3+	3+	3+
Eye.....	2+	+	5+	5+	5+
Stomach.....	−	−	4+	4+	5+
Muscle.....	2+	+	4+	4+	5+

* Ten per cent suspension of segments stored at 0 C for 8 days.

† Relative growth compared to broth control: − = no growth; values greater than those of control indicate growth enhancement.

stomach areas they were squashed between a Millipore filter pad and a Millipore membrane and the test organisms were streaked toward the krill areas. Three of the five immature forms tested also inhibited gram-positive bacteria. It is interesting to note that in the absence of inhibitory material from the phytoplankton, the krill material usually enhanced bacterial growth and pigmentation.

The antibacterial activity of the lobster krill appeared to be in their phytoplankton laden stomachs. In order to determine if sea water phytoplankton exerted a differential antibacterial activity, sea water and plankton net samples were obtained at the hydrographic stations made by the *San Martin* in the Weddell Sea. The phytoplankton in six water samples was concentrated on hydrosal assay Millipore membranes which were rinsed with sterile physiological saline or autoclaved, filtered sea water, placed algae side down on Eugon broth soaked pads, and streaked on the upper sterile surface with the test bacteria. The phytoplankton concentrates from 1 to 500 ml of three of the six samples tested were inhibitory. On three occasions the phytoplankton was so dense that the plankton net was covered by a bright green jelly-like material which appeared to consist almost exclusively of Halosphaera algae. Dry weight determinations were between 4 and 6 per cent dry matter. Serial 10-fold dilutions of the fresh material were made in Eugon broth and 1-ml aliquots were concentrated on the Millipore membranes and treated in the same manner as the concentrates from the water samples. The weakest preparation obtained inhibited the gram-positive test organisms at 0.01 per cent and the gram-negative at 1.0 per cent wet weight concentrations. The results on the most active plankton net sample are given in figure 2. As in the other samples, the gram-positive bacteria were more susceptible than the gram-negative test organisms. This material was active at a concentration of 0.0001 per cent or 100 parts per million on a wet weight basis. On a dry weight basis this would be 5 ppm which indicates that an extremely potent antibacterial substance is present in some phytoplankton samples. As the algae became more concentrated,

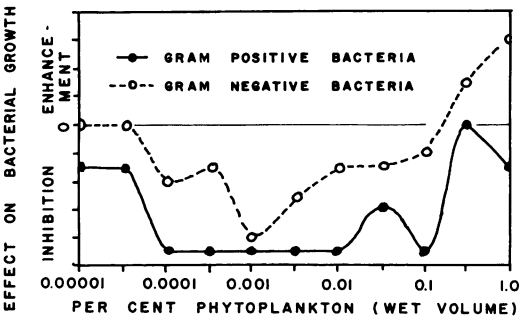

Figure 2. The effect of antarctic sea water phytoplankton on bacterial growth.

the antibacterial activity was lost and the material even became growth enhancing.

DISCUSSION

One of the main bacteriological questions posed by previous investigations in the Antarctic was the existence of "bacteriologically sterile" birds. Five of the six expeditions reported, by species, birds from which bacteria could not be cultivated. McLean (1919) proposed that the food of these birds was sterile. Bunt (1955) suggested that yeasts or protozoans might be causing complete inhibition of bacterial growth by direct competition for nutrients or the production of antibiotic substances. McBee (1957, *unpublished data*) cultured the intestinal contents of three birds, found growth to be plentiful in agar shake tubes and absent from surface cultures, and concluded that the earlier reports on the sterility of the intestinal contents of antarctic birds were based upon inadequate observations. Of the sixteen birds studied in detail in this study, four did not yield a detectable aerobic flora. In the scope of earlier work in which anaerobic procedures were crude or were not used, these birds might have been regarded as being "bacteriologically sterile." However, an anaerobic intestinal flora was present in all birds studied. The proventriculus and ventriculus contents of some birds were free of a cultivable flora, either aerobic or anaerobic. Gram stained smears from these materials indicated an abundant bacterial flora. The presence of bacterial forms in food material from which neither aerobic nor anaerobic bacteria could be cultivated suggested the presence of strong antibacterial substances in the diet of these birds. A procedure was developed to estimate the bacterial activity of nonsterile substances in order to determine if antibacterial activity of penguin tissues in the giant fulmar's proventriculus contents, in which bacteria were present but did not grow, indicated that tissue fluids were exerting a bacteriostatic or bactericidal activity. The antibacterial activity of normal serum is well known and has been recently reviewed by Skarnes and Watson (1957). Assays on the antibacterial activity of blood serum from twelve antarctic birds indicated that all were inhibitory at a 1:20 dilution and five were inhibitory at a 1:160 dilution. Assuming that these birds, like chickens (Sturkie, 1954), have approximately 8 per cent blood volume and that serum constitutes 50 per cent of the blood volume, then a 4 per cent suspension or 1:25 dilution of serum would occur in a macerated bird. Therefore, there appeared to be a sufficient concentration of inhibitory substances in serum to explain the antibacterial activity of the ingesta of predatory birds. The antibacterial activity of the penguin tissues in the giant fulmar appeared to not only inhibit its gram-positive anaerobic flora, but to eliminate any possible gram-negative aerobic flora. A differential antibacterial activity of normal food materials may be responsible for the apparently aerobe-free birds. This hypothesis is strengthened by the observation that all four birds free of detectable aerobes had an anaerobic flora that consisted of one morphological type.

Antibacterial activity in the gastric contents of some of the penguins feeding on lobster krill drew attention to the green discoloration of the proventriculus and ventriculus lining of these birds. The antibacterial activity and greenish material in the lobster krill (Euphausian shrimp or *Euphausia superba*) was found to be in their phytoplankton laden stomachs. Samples of sea water phytoplankton also exhibited marked antibacterial activity. Sea water phytoplankton, freshly caught krill, and the gastric contents from penguins possessed a similar differential antibacterial activity. Gram-positive bacteria were apparently more susceptible than gram-negative bacteria. This differential antibacterial activity against gram-positive bacteria may explain the predominant gram-negative microflora in antarctic birds which possess an aerobic flora.

The antibacterial property of sea water phytoplankton may affect the bacterial population and ecology in surface sea water. A heat-labile, filterable, bactericidal factor of sea water for sewage organisms was observed by ZoBell (1936). Studies on the viability and dispersal of coliform bacteria in the sea (Ketcham *et al.*, 1949; Vaccaro *et al.*, 1950) also indicated that a bactericidal factor was present in sea water which was heat labile, increased during the aging of sea water and underwent a seasonal variation. Harvey (1955) states "there is some natural brake upon the growth of planktonic bacteria in the open sea" and "it is an outstanding question why

offshore sea water, which contains sufficient nutrient for the production of several million bacteria per cubic centimetre . . . normally supports a population of no more than 10–200 bacteria per cm.^{3} as free living planktonic cells." The fresh water algae chlorella was found by Pratt *et al.* (1944) to liberate an antibacterial substance chlorellin in poor yield which was found by Spoehr *et al.* (1949) not to be in the fresh cells but to result from the photooxidation of unsaturated fatty acids. Steemann Nielsen (1955) reported one trial in which a dilute chlorella culture with its accompanying bacterial population consumed less oxygen in light than in dark bottles. On the basis of data collected by another investigator which indicated a variable rate of oxygen consumption in dark bottles taken in the Sargasso Sea, Steemann Nielsen postulated the existence of antibiotic production by planktonic algae.

During the assay of sea water and phytoplankton samples in the Weddell Sea, water samples containing algae remained clear when left exposed to the air, while aliquots of the same sample which had the algae removed by Millipore membrane filtration became very turbid and formed a large sediment of bacterial cells upon standing. The ability of fresh algae to inhibit both gram-positive and gram-negative organisms in very small quantities (five parts per million dry weight) indicates that a strong antibiotic-like substance which is water soluble can be liberated by certain species of algae. As in the chlorella experiments (Pratt, 1948; Steemann Nielsen, 1955) a higher antibacterial activity was observed in lower than in higher concentrations of algae. This substance was heat labile and filterable and may be the bactericidal substance observed by ZoBell (1936), Ketcham *et al.* (1949), and Vacarro *et al.* (1950). The marked activity of fresh preparations indicated that this material was different from chlorellin. Since all antarctic birds, animals, and fishes live either directly or indirectly on phytoplankton, this material may play a large role in the bacterial ecology of antarctic fauna. This phenomenon may not be restricted to the dense phytoplankton areas of the antarctic.

The predominant gastrointestinal microflora of domesticated birds consists of lactobacilli, coliforms, and the enteric group of streptococci, in that order, (Sieburth *et al.*, 1951, 1952, 1954) and appears to reflect the microflora of the feed. Lactobacilli and the enteric group of streptococci were not detected in the antarctic birds studied. Slow lactose fermenting strains of *E. coli*, other slow or lactose nonfermenting enterobacteria, streptococci, other than the enteric group, and diphtheroid-like organisms were the predominant bacterial types found in the birds with an aerobic flora. The fastidious diphtheroid-like and gram-negative rods and the anaerobic organisms died out on subculture and were not studied. *Proteus mirabilis*, *P. rettgeri*, *P. vulgaris*, and *Alcaligenes* species were found in low numbers in some birds. Of the five cultures from antarctic birds brought back by Dr. Charcot, Mlle. Tsiklinsky (1908) identified *Bacillus pyocyaneus* obtained from a penguin and *Bacillus megaterium* which was found in both a sea gull and a cormorant. Bunt (1955) observed *E. coli*, *Bacillus* species, nonsporing rods, and micrococcus. Since most descriptions of bacteria from antarctic birds are morphological and incomplete it is almost impossible to compare bacterial types observed by the various workers.

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SUMMARY

During the 1957–1958 Argentine Antarctic Expedition through the South American quadrant of Antarctica, microbiological studies were conducted on antarctic birds in an improvised bacteriological laboratory aboard the icebreaker *ARA General San Martin*.

Twenty eight birds were studied, sixteen in detail. "Bacteriologically sterile" birds were not observed; in the four birds in which an aerobic flora was not detected, obligate anaerobes were found. In the remaining twelve birds which had an aerobic flora, slow or negative lactose fermenting gram-negative rods were the predominant bacterial types.

Lactobacilli and the enteric group of streptococci were not detected but other species of streptococci and diphtheroid-like organisms were present in good numbers in some birds. Typical rapid-lactose fermenting strains of *Escherichia coli* were not observed in the penguins (*Pygoscelis* species) feeding on lobster krill and delayed lactose fermenting strains were more prevalent in the flying birds.

The strong antibacterial property of blood serum in some of the antarctic birds appeared to be the factor responsible for modifying the gastrointestinal flora of a predatory giant fulmar.

Algae in sea water phytoplankton had a marked differential antibacterial activity. This observation not only explains the growth inhibiting factor in the gastrointestinal material of penguins feeding on the algae laden lobster krill but may also explain in part the low planktonic bacterial population in sea water and the predominance of gram-negative organisms.

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